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DAIRY BACTERIOLOGY

DAIRY BACTERIOLOGY

Bernard W. Hammer, Ph.D.

Third Edition

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PREFACE

To Third Edition

The third edition of "Dairy Bacteriology" covers the same general field as the earlier editions, although there are minor rearrangements of the material. Some sections have been expanded and others contracted, depending primarily on whether the subjects involved are receiving more or less attention than formerly. In some instances this attention has been influenced by the war; for example, the heavy production of milk powder for direct and indirect war purposes resulted in extensive bacteriological studies on milk powder that provided much useful information. In other instances increased attention to a subject has been the result of definite recognition of its importance; for example, the importance of the bacteriological quality of dairy-plant water supplies has been shown by the outbreaks of spoilage such supplies have caused in various dairy products, especially butter.

As in the earlier editions, only special laboratory methods are included, and "Standard Methods for the Examination of Dairy Products" (last edition) should be used for the procedures given there. The changes that have been made in methods which at one time were thought to be thoroughly standardized show that the last edition of a publication giving a laboratory procedure should be followed faithfully.

In the general field of dairy bacteriology there remain numerous important problems the solution of which would be very much worth while. The control of objectionable numbers and types of organisms in dairy products is simple, and this suggests that all the problems in dairy bacteriology are in the same category. Actually, many problems along such lines as development of the desired flavors in certain products through the action of organisms or economic production of various fermentation materials from milk solids that would otherwise be wasted are very complicated, and the solution of any one of them constitutes a real accomplishment.

Many persons have aided greatly in the revision of "Dairy Bacteriology" and their help is gratefully acknowledged. Special thanks are due Miss Elise Hoffman of the Golden State Company library, Mr. R. W. Orr of the Iowa State College library, Dr. R. V. Hussong of the University of Illinois, and Dr. H. F. Long of the Sugar Creek Creamery Company for supplying reference material, reviewing sections of the manuscript, etc.

B. W. HAMMER

Sarasota, Florida

PREFACE

To First Edition

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The material presented in the following pages represents that included in the beginning course in dairy bacteriology at Iowa State College. This course follows one in general bacteriology and is intended primarily for students in the course in dairy industry but is also required of students in dairy husbandry and is elected by others specializing in bacteriology. Four recitation-lecture periods are held each week for one quarter of about twelve weeks. Assigned readings intended to supplement the material presented and to give the students a more detailed knowledge of important points than the class room permits are also required. The laboratory work consists of three two-hour periods each week and the exercises are selected with the idea of illustrating the more important procedures used in dairy bacteriology. Considerable time is spent on the milk fermentations since a good understanding of these is believed to be fundamental to the practical knowledge of dairy bacteriology which is so essential to the technical dairyman. Some of the organisms studied are isolated by the students themselves from milk showing various changes. This illustrates the distribution of the organisms studied better than can be done in any other way and also adds interest to the work; the continued study of supplied cultures tends to give the students the impression that bacteria exist mainly in laboratory cultures.

The relative importance, from the standpoint of the class room, of the various phases of dairy bacteriology is a question on which considerable diversity of opinion is to be expected. The proportion maintained in the following presentation has been found to be satisfactory under the conditions existing at Iowa State College and satisfactorily to relate dairy bacteriology to the courses that follow it.

Material on the standard methods for the bacteriological examination of milk is not included because it is so readily available elsewhere in a form adapted to laboratory use. For the most part, descriptions of organisms are omitted because brief descriptions are included in books dealing with determinative bacteriology and detailed descriptions can be best studied in the original reports.

B. W. HAMMER

DAIRY INDUSTRY BUILDING
Ames, Iowa

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Introduction

Importance of Dairy Bacteriology

The need for knowledge of the bacteriology of dairy products is shown by three distinct considerations:

1. Spread of diseases through dairy products. Investigations extending over many years have proved that diseases in both man and animals sometimes are caused by the pathogenic organisms spread by milk and its derivatives. Although the presence of such organisms is not peculiar to food materials from the dairy but occurs with products from a variety of sources, it is especially serious with dairy products because they frequently are consumed without being heated and thus without destruction of harmful organisms that may be present. Accordingly, knowledge of the sources of pathogenic organisms in dairy products, conditions under which they grow, and methods of keeping them out or destroying them when they gain entrance is essential. Such knowledge is important to the producer or manufacturer of dairy products, because of the responsibility of selling only safe foods, and it is of extreme importance to the inspector of dairy plants and dairy products since inspection is intended to represent the final protection to the consumer. Although the producer or manufacturer and the inspector should guard the consumer's interests, it is desirable that the consumer be sufficiently informed to decide whether a particular system of handling a dairy product is satisfactory. In the same way, the farmer or other individual using milk or milk products for the feeding of animals should have the knowledge that will make possible a reliable decision as to the probability of such materials introducing diseases into his herds and flocks.

2. Deterioration and spoilage of milk and its derivatives. Many micro-organisms, as a result of their growth, cause undesirable changes in milk and its derivatives and, accordingly, are responsible for deterioration and spoilage. The producer of milk and cream should know the sources of organisms causing rapid changes in these materials, conditions favoring their growth, and methods of eliminating them or preventing their activity, since the income obtained largely depends on the quality of the products supplied. The manufacturer of milk derivatives must contend with problems similar to those of the producer of milk and cream and additional ones also, since butter, cheeses, etc., frequently are stored for considerable periods, during which there may be unusual opportunities for a decrease in quality. The problem of deterioration and spoilage is especially important with the cheeses requiring ripening, since conditions must be favorable for growth of certain desirable micro-organisms and these may allow development of undesirable species.

3. Development of desirable micro-organisms in certain dairy products. The pleasing flavor of some dairy products, such as butter, cheeses, and fermented milk, and the desired texture of many cheeses are due largely to the development of certain micro-organisms during manufacture and ripening. An understanding of the action of these organisms, conditions favoring their growth, etc., is important and very necessary if products having a uniformly high quality are to be produced and marketed. The manufacturing methods used for certain dairy products were developed empirically before the role of micro-organisms was understood, but various valuable improvements have resulted from knowledge of the action of the important species of organisms and progress along this line undoubtedly will continue.

Chapter 1

Bacterial Counts on Milk

Studies on the numbers of bacteria in various materials quickly followed development of methods applicable to such determinations. In investigations on milk the bacterial content was found to be an indication of the general conditions of production and handling and of the keeping quality, and counts soon were employed in obtaining information along these lines. Determinations of the numbers of bacteria also have come into extensive use in studies on the roles of various organisms in both the desirable and undesirable changes occurring in dairy products.

Routine counts and research counts. Bacterial counts on milk and certain of its derivatives are needed for two rather distinct purposes. *Routine* counts are used in the grading of milk and in the general control of milk supplies; *research* counts are employed in investigations involving the action of organisms on dairy products. With routine counts the results must be obtained cheaply and quickly. With research counts accuracy under specific conditions is the principal consideration, and expense and the period required are not such important factors. Standardized methods are necessary with routine counts so that results obtained in different laboratories, or in one laboratory at different periods, can be compared, and the significance of various counts interpreted on the basis of general experience in milk-control work. It is not necessary for a routine count to give the actual number of bacteria in a sample, but rather that a representative portion be determined. The bacterial limits set by ordinances and regulations regularly recognize that routine methods of examination do not necessarily give as high counts as might be obtained by special methods. Research counts are obtained under widely differing conditions, depending on the particular object in view, and it is important that the exact procedure be clearly given in any statement of results.

ROUTINE COUNTS

Two methods commonly are employed in the routine counting of the bacteria in milk, the agar plate method and the microscopic count or direct microscopic method. The procedures used with these methods have been well standardized for use in the supervision of milk supplies.[1]

Plate method. The introduction of liquefiable solid media afforded a means of determining the numbers of bacteria in various materials by mixing a given weight or volume with the liquefied medium in plates, allowing this to solidify, and then counting the colonies that develop on incubation. The procedure has been widely used in the dairy industry and has found extensive application in the examination of milk.

The medium used for plating milk, the temperature and period of incubation, and other variations in the procedure have an influence on the numbers of colonies developing, through their general effect on the growth of organisms. Recognizing the necessity of standard methods for the bacteriological examination of milk, if various results are to be directly comparable, the American Public Health Association, in 1905, appointed a committee to study the various methods used and to recommend a uniform procedure. Preliminary reports were made, a complete report was published in 1910, and additional reports have followed from time to time. The more recent reports represent the ideas of various organizations interested in milk supervision. In using the plate method for routine bacterial counts on milk it is essential that the standard procedure be followed in detail[1] because of the influence of various factors on the results. Black[2] surveyed milk laboratories in the United States and found that with agar plate counts each item required by standard methods had been adhered to by several laboratories but that practically every item had been neglected by one or more laboratories. At the time of the survey not one laboratory actually met all requirements for equipment and procedure, although a very few approached them.

Objections to the plate method. Objections have been raised to the plate method of determining the numbers of bacteria in milk as a means of controlling milk supplies. The more important of these objections are:

1. *Pathogenic organisms are not detected.* Because the most important reason for controlling a milk supply is to prevent spread of diseases through it, failure of the counts to detect pathogens constitutes a definite defect. These organisms are not detected because certain of them (for example, *Mycobacterium tuberculosis*) do not grow under the conditions provided, and because those that do grow (for example, *Eberthella typhosa*) cannot be distinguished from non-pathogenic species by the colony appearance. If a rapid and accurate method for the

determination of pathogens in milk ever is devised, it undoubtedly soon will supplant routine bacterial counts to a large extent.

2. Counts are not accurate. Counts obtained by the plate method do not represent the actual numbers of bacteria present because of various factors. None of the known media are satisfactory for all the species that may be in milk. The air supply in plates prevents growth of some types. The temperature is not suitable for certain organisms. Many of the colonies come from pairs or groups of cells rather than one cell. Accordingly, the values obtained with the plate method are lower than the actual numbers of bacteria present; extent of the discrepancy is dependent mainly on the species in the milk. The fact that the plate count is lower than the actual number of bacteria in a sample of milk is of no great consequence in control of milk supplies, since an allowance is made for this in the establishment of bacterial standards; however, the method used should give a fairly constant percentage of the total numbers present.

In some instances large discrepancies [3,4,5] have occurred in results obtained by various laboratories on milk from the same lot. These may have been due to variations in the media used, in the periods between diluting the milk and pouring the plates, in the methods of incubating the plates, and in other factors, all of which are of importance. Differences in the results obtained by persons working in the same laboratory also have been reported. Often two or more counts that show considerable variation lead to the same general conclusion with reference to the care used in production and handling of the milk so that the variation is of little practical importance. In many trials the agreement between results obtained in different laboratories is surprisingly close, when consideration is given to the fact that development of microscopic organisms is involved.

Tiedeman [6] reported that standard plate counts made by different laboratories on identical samples of low-count raw milk are in close agreement. Such counts by different laboratories on high-count raw milk, on abnormal raw milk, and on pasteurized milk show marked variations. He also noted that the preponderance of standard plate counts falling out of range is on the low side, suggesting failure of some of the bacteria to produce discernible colonies.

3. Specific information regarding species is not furnished. With most organisms the colony appearance does not identify the species. If the organisms in a sample of milk could be classified from the colonies that develop on agar, valuable information would be available. However, failure to obtain definite information regarding species cannot be considered a serious objection to the plate method, since the counts are made primarily to determine the general conditions of production and handling of the milk.

4. Period required before results are available is too long. Because of the period required for incubation of the plates, data on the number of bacteria in a sample of milk ordinarily are not available until the milk has been consumed. The quality of a milk supply, however, cannot be determined by one bacterial count, and it is only when results of a series of counts are available that a fair idea of the character of a supply can be formed. A single high count does not justify exclusion of the lot of milk on which it was obtained if the counts on the preceding lots from the same source are satisfactory; it simply indicates an undesirable condition that should be located and corrected at the earliest possible moment. When the real significance of a single high count is recognized, it is evident that consumption of milk before the count on it is available does not constitute an important objection to the plate method.

Microscopic count. Methods of counting bacteria in various materials by means of a microscope have been employed since the early days of bacteriology and eventually the idea was applied to milk. Some of the procedures consisted of preparing a film from material thrown out of the milk with a centrifuge and then staining and examining it, but there is only a partial recovery of the organisms with them. The method now commonly employed involves spreading 0.01 ml of milk, measured with a capillary pipette, or under certain conditions with a calibrated loop, over an area of 1 sq cm on a slide and, after the milk is dried and the fat removed, the fixing, staining, and examination of the resulting film.[1] A combined solvent, fixing, and staining solution often is used, particularly under field conditions, and is very satisfactory for raw milk. Counts are made on two bases, (1) individual cells, and (2) groups of cells in which unseparated clumps, chains, and pairs of cells and individual cells are counted as units. The latter procedure attempts to make microscopic counts more nearly comparable to plate counts in which colonies often come from groups of cells. The procedure outlined should be followed exactly for dependable results. Black[7] found that the requirements of standard methods were rather frequently violated by milk laboratories in the United States.

Special counting procedures. Hanks and James[8] concluded that the natural distribution of bacteria in films prepared for microscopic counts is not taken into consideration when square films are used or random observations made; preparation of circular films and microscopic sampling along two diameters at right angles take into account the concentric distribution of bacteria and minimize the effect of abnormal distribution.

Olson and Warren [9] developed an instrument for marking circles on glass slides so that circular films can be prepared easily. They also modified the mechanical microscope stage so that a series of equally spaced fields across the diameter of the circular film can be counted. Buck [10] likewise developed a microscope field spacer; with it, counts are automatically attained in increments of ten equally spaced fields.

Advantages of the microscopic count. The microscopic count has certain advantages over the plate count, the more important of which are:

1. *Results are quickly available.* With the microscopic method a count can be obtained in a very short period, provided the microscope has been standardized and the reagents prepared; this is particularly advantageous in connection with samples brought to laboratories by producers, since such persons usually wish the results at once and often are interested in seeing the bacteria in the milk.

2. *Less work is required.* The number of samples that can be examined in a given period with the microscopic method is relatively large, owing mainly to the fact that preparation of the medium and dilution bottles, washing and sterilization of the glassware, etc., are not involved. The actual number is influenced primarily by the number of fields counted per sample. If lots of milk are simply divided into grades, as in certain types of inspection work, they can be handled very rapidly; high-count samples often can be graded by examination of relatively few fields.

3. *Less apparatus is required.* The amount of apparatus necessary for the microscopic count is relatively small, a microscope being the only expensive item. With the plate method the investment in incubators, sterilizers, and glassware is comparatively large.

4. *Less expensive.* Since the microscopic count requires less work and apparatus than the plate method, expense per sample also is less. If bacterial counts are to be used extensively, the lowest possible cost is advisable because the funds available often are limited. With only a small number of bacterial counts on a given milk supply, the conclusions drawn may be open to criticism.

5. *Microscopic preparations give a permanent record.* The preparations used in making microscopic counts can be kept and examined whenever, within a reasonable period, a question arises with reference to the counts. Permanent records are especially important in connec-

tion with certain points, such as morphology of organisms, that are difficult to report and not easily included in a statement of results.

6. *Some idea of the species present can be obtained.* Although no definite information regarding species can be obtained from microscopic observations alone, some idea of the general types of bacteria present can be secured by persons familiar with the organisms common in milk. A predominance of the ordinary lactic acid bacteria is rather easily determined from microscopic preparations, and some of the organisms coming from infected udders can be recognized with considerable accuracy. Information on the types of organisms frequently is valuable in helping to determine the cause of high bacterial counts on certain samples.

7. *Numbers of body cells present can be determined.* The stained preparations employed in the microscopic method permit the counting of leucocytes and other body cells in milk, and unusually large numbers are evident from superficial examination. Although the significance of these cells, even in rather large numbers, may be questioned, excessive counts indicate abnormal udder conditions, particularly when they are associated with large numbers of streptococci. The analyst experienced in the microscopic method frequently can detect abnormal udders by the microscopic picture of the milk.

8. *Preservatives can be used with samples.* Milk samples intended for microscopic examination can be held for short periods with preservatives, such as formaldehyde, because the bacteria need not be kept in a living condition. Samples so treated cannot be held for very long, however, since the organisms killed by the preservative soon begin to autolyze and this seriously interferes with their staining. If the preserved samples are allowed to stand until the cream is very compact, difficulty is experienced in redistributing it.

Disadvantages of the microscopic count. In addition to its advantages, the microscopic count has certain disadvantages compared to the plate count. The more serious of these are:

1. *Irregularities are inherent in the procedure.* Results obtained with the microscopic count are influenced by the irregularities inherent in it. Organisms often are very unevenly distributed in a smear. In some smears many fields show comparatively few or no bacteria but an occasional field shows a clump that may contain up to several hundred cells; under these conditions the results are greatly influenced by the number of clumps that happen to be encountered in the fields observed. The milk equivalent of the portion of the smear examined is relatively small and may not be representative of the sample under study. Smears

vary in thickness so that a field does not always represent exactly the same volume of the original milk. There is considerable evidence that not all the bacteria in a smear stain well enough to permit their detection. Some bacteria that do stain may not be recognized as organisms, especially with low magnification. Because of these irregularities, there may be considerable variation between duplicate counts on a sample of milk.

Levowitz[11] suggested that instead of observing a limited number of fields in a smear, a sufficient area be examined to include enough cells to permit corroboration on re-examination. His method involves counting one or more strips across a smear, using a field diameter of 0.200 mm.

2. *Less generally useful for low-count milk.* Irregular distribution of organisms in a smear is especially significant with low-count milk. The microscopic count can be used with such milk but it is a laborious procedure if reasonably detailed results are desired; simple detection of relatively low-count milk is not particularly time-consuming. With aseptically drawn milk, Strynadka and Thornton[12] concluded that the milk observed in the examination of 60 fields seldom was reasonably representative of the sample from which it came; examination of 1,000 fields sometimes gave misleading results. They noted that in milk with a low bacterial count 0.01 ml cannot be depended upon as being truly representative.

3. *Less generally useful for pasteurized milk.* When a microscopic count is made on pasteurized milk a portion of the organisms that have been killed by the heat still take the stain. This limits the usefulness of the method in the supervision of city supplies and in checking operations in pasteurizing plants. It appears that the percentage of killed cells which can be seen varies with the species present and also with the stain used, light adjustment of the microscope, care used in making the observations, etc.; with selected stains, more of the cells can be seen than with those commonly used on raw milk.

Hastings and Davenport[13] noted that as a result of pasteurization at 62.8 C (145 F) for 30 min there was a decrease in the numbers of bacteria detected by the microscopic method which, in some instances, was very large. The decrease often was more after 6 hr at 10 C (50 F) than immediately after heating, and occasionally there was a further decrease after 24 hr. The investigators also found that pasteurization of low-count milk inoculated with pure cultures commonly gave results similar to naturally contaminated milk, although with some of the species there was no apparent change in the staining or the number of cells.

In the data reported by Macy [14] the comparative microscopic counts, and also plate counts, on milk before and after pasteurization indicate that many of the organisms killed by pasteurization still are stainable. He noted that the types of bacteria in raw milk have a great deal to do with the results obtained on microscopic examination of pasteurized milk and that, if used with discretion, such results serve the control laboratory effectively, especially when microscopic examination of the raw milk has been practiced. Stark and Campbell [15] concluded that with the microscopic count it is not always possible to distinguish with certainty between living and dead bacteria.

In some cases attempts are made to distinguish between living and dead bacteria in smears from pasteurized milk by the intensity of the staining. However, there are variations in the staining intensities of the organisms in smears from raw milk so that conclusions are open to question.

Comparison of microscopic and plate counts. A microscopic count on a sample of milk commonly is significantly higher than a plate count. Robertson [16] found the most frequent ratio between results of microscopic and of plate counts, when the plates were incubated 2 days at 37 C (98.6 F), was 4 to 1; this is the ratio commonly recognized and is the one used when it is necessary to calculate results of one count in terms of the other. Actually, the ratio varies widely from one lot of milk to another and the 4 to 1 relationship is only rarely met. In general, the difference between the results of the two methods is relatively large with low-count milk and relatively small with high-count milk. This is due, in part, to the fact that in low-count milk the organisms are mainly those that have come from various sources and include types that will not grow on the agar used for plating, whereas in high-count milk the organisms largely represent those that have grown in milk, many of which are capable of developing on the agar. There is a tendency also for a greater percentage of the bacteria to be in clumps in low-count milk than in high-count milk.

In making microscopic counts, groups of bacteria (clumps, chains, pairs, etc.) sometimes are counted as units along with isolated cells in an attempt to have the results in closer agreement with the results of plate counts and, normally, this reduces considerably the ratio between the results of the two methods. However, no such simple procedure eliminates the difference because of the various factors involved and it introduces into the microscopic count one of the causes of the low values obtained with the plate method. The average size of the groups of bacteria in milk undoubtedly varies with the species present, and also with the conditions under which growth occurs. Baker *et al.*,[17] in their study of the relation between lactic acid production and bacterial growth in the souring of milk, noted that the average size of the groups of lactic acid organisms in milk was 1.8 individuals. Whiting[18] found the average size of the clumps of bacteria in 3,480 samples of market milk from the Geneva, N. Y., supply was 11.1 individuals, while in 150 samples from the Ithaca, N. Y., supply it was 4.1 individuals; the word *clump* was used to refer to isolated cells as well as to groups.

Significance of routine counts on raw milk. Routine bacterial counts are widely used to determine the general sanitary quality of raw milk. If milk is produced under unsatisfactory conditions, if it is kept warm, or if it is held at satisfactory storage temperatures for more than an acceptable holding period, high bacterial counts commonly result. Occasionally, certain udder conditions are responsible for large numbers of bacteria in milk. A routine bacterial count on a sample of milk accordingly gives information along four lines as follows:

1. Care used in production. The care used in production of milk, especially with reference to such factors as condition of the utensils and cleanliness of the coats of the cows, is reflected in the bacterial count of the freshly drawn milk. However, even with very insanitary methods of production the counts are not surprisingly high when the milk is first drawn; they only rarely exceed 1,000,000 per milliliter and commonly fall below 500,000. It is the multiplication of organisms gaining entrance to the milk, rather than the contamination, that is responsible for the very high counts sometimes obtained.

2. Temperature of holding. The rate of multiplication of bacteria in milk is influenced more by the temperature of holding than by any other factor. At favorable temperatures growth is surprisingly rapid, and excessive numbers of organisms quickly develop. Very high bacterial counts on milk more often are due to holding temperatures that are especially favorable for multiplication of bacteria than to any other cause. The influence of a particular temperature on the rate of growth varies widely with different species. A consideration of importance in connection with the temperature effect is the history of the milk. Other things being equal, aged milk commonly shows more active bacterial multiplication than fresh milk when the two are held under the same conditions.

3. Period of holding. The period of holding is another important factor influencing multiplication of bacteria in milk and one that naturally is related to the temperature of holding. As short a period as

1 hr may account for an increase in the number of bacteria present in milk if the holding temperature is very suitable for growth, whereas longer periods are required for significant increases as the temperature becomes less favorable. Temperatures that satisfactorily check bacterial development for 24 hr may not do so over more extended periods. If milk is held long enough, multiplication of bacteria occurs at all the usual temperatures above the freezing point, and accordingly temperatures approximating 0 C (32 F) do not justify excessive holding, although they prevent the multiplication of bacteria for considerable periods.

4. Condition of the udder. High bacterial counts on milk may be obtained as a result of the presence of large numbers of bacteria in the milk at the time it leaves the udder. Frequently, such milk comes from infected udders but it also may come from udders that are apparently normal. There is no close correlation between infected udders and high counts on milk as it is drawn, and in some instances milk coming from such udders has an unusually low bacterial count.

Information given by routine bacterial counts along the four lines considered is suggestive rather than definite. When a high count is obtained it usually is more or less difficult to decide which of the various factors is responsible, although laboratory experience often makes it possible to give a reasonably accurate opinion; a variety of bacterial types suggests extensive contamination, many colonies of the common lactic acid organisms indicate growth at temperatures higher than those usually advocated for holding milk, etc. Frequently, high counts are due to a combination of factors and determination of the cause then is more complicated than when only one factor is involved. Although the history of a sample of milk, as determined by the number of contained bacteria, is not as definite as is desired, defective methods of production and handling often can be detected and, accordingly, bacterial counts are indispensable in the proper control of milk supplies.

The relatively low cost of bacterial counts is an important consideration and it is generally recognized that, for the expense involved, bacterial counts give more information regarding methods of producing and handling milk than any other procedure. Bacterial counts are extensively employed by milk distributors as well as health departments and other supervising agencies. A bacteriological laboratory is an important part of many modern dairy plants and, in some of these, large numbers of bacterial counts are made on the milk as it comes from the producers; in certain instances a premium is paid for milk of high sanitary quality as shown by these counts or other tests. An examination of the milk also is commonly made at various stages during the handling in the plant in order to check the methods employed.

Significance of routine counts on pasteurized milk. When applied to pasteurized milk, plate and microscopic counts give information along somewhat different lines.

Plate counts are valuable in the supervision of supplies of pasteurized milk, since they aid materially in controlling the efficiency of both the pasteurization and the handling of the milk after heating. Pasteurization temperature and time records are important, but there is no assurance that all the milk has been heated under the recorded conditions and, moreover, the value of efficient pasteurization may be more than counterbalanced by careless handling subsequent to this treatment. In some cases also the raw milk contains large numbers of heat-resistant organisms that can best be detected with plate counts.

Even with the irregularities in the staining of the bacteria in pasteurized milk, microscopic counts supply valuable information with reference to the numbers of bacteria present before heating and also help to explain unsatisfactory pasteurization by showing the general morphologic types of bacteria present; this is particularly true when special staining procedures are employed.

In conjunction with other tests, microscopic counts on pasteurized milk are of increasing importance.[19,20] A program of (1) phosphatase tests to determine adequate heating of all the milk, (2) tests for coliform organisms to detect recontamination of the heated milk, and (3) microscopic counts to establish the bacteriological quality of the original milk provides a relatively simple means of controlling a milk supply. Such a procedure is inexpensive and furnishes information that is very useful to control officials and milk distributors in providing safe milk.

Criticism of routine counts. Bacterial counts have been criticized as being of little value in determining the safety of a milk supply, since the great majority of the organisms present in milk are harmless. *Streptococcus lactis* is the most common species in milk having a high bacterial count, and it is consumed in large numbers in such products as buttermilk, cheeses, and butter that are regarded as valuable foods. However, conditions which permit entrance of many organisms, or growth of those that do gain entrance, are the conditions most likely to result in introduction or multiplication of pathogens so that, on the average, the lower the count the less the danger of harmful organisms being present. A high bacterial count is no indication that milk is harmful and, on the other hand, a low count is no guarantee that milk is safe, but the conditions of production and handling that give high counts are more likely to result in the presence of harmful organisms than conditions yielding low counts. There is a great difference in the importance of organisms from various sources, a few from an infected human throat, for example, being much more objectionable than a larger number from the flanks of the cows or the utensils, and total bacterial counts do not take this into consideration. However, such counts are an indication of the care used in production and handling of milk and suitable methods are necessary in providing a satisfactory milk supply. The ideal examination would be one that determines the dangers from the various organisms present and, when such a method of examination is devised, it probably will supersede bacterial counts. There are many difficulties involved in such a determination and it is inadvisable to discard the present method, with its admitted defects, until something better is available with which to replace it.

In some instances milk pasteurized at satisfactory exposures has a high plate count immediately after heating, owing to heat-resistant organisms in the raw milk or to multiplication of certain bacteria in the operating pasteurization equipment. Such counts have been advanced as an objection to the use of bacterial counts in arriving at the sanitary quality of milk on the theory that they give the wrong impression of the treatment of the milk. However, many heat-resistant organisms in the raw milk, or extensive development of organisms in the equipment, should be detected and corrected; they can be avoided easily with proper precautions.

Bacterial standards. Use of bacterial counts for the control of milk supplies resulted in the establishment of bacterial standards. In 1900, the New York City Department of Health attempted a standard of 1,000,000 per milliliter, but it was found practically impossible to enforce at that time because of the complicated situation in the milk supply. In 1905, the Boston Board of Health legalized a standard of 500,000 per milliliter. At present many cities have bacterial standards for milk. They vary somewhat because of the different conditions under which the supplies are produced. Also, different grades of milk commonly have different bacterial standards. The standard for cream often is higher than that for milk of the same grade, since during skimming considerable numbers of bacteria may be carried into the cream by the clumps of fat globules.

Special grades of milk have very low bacterial limits. The usual standard for certified milk is 10,000 per milliliter, plate count. From this value the standards become more and more lenient from one grade to another of milk for direct consumption. If the milk is to be pasteurized, it has a higher standard than if it is to be consumed raw because of the protective action of the pasteurization. In general, standards are becoming more and more rigid, as improvements are made in milk production and handling procedures; standards now readily met would have seemed impossible to achieve in the early days of milk control. In some areas milk for manufacturing purposes must comply with bacterial standards. Commonly, they are rather lenient.

In certain instances, dual standards, which involve one limit for the warm months and another for the cold months, are used; this is simply a recognition of the fact that it is more difficult to control the bacterial content of milk in summer than in winter.

In the establishment of a bacterial standard for milk it is necessary to state whether it refers to the plate or the microscopic count because of the difference in the results obtained with the two procedures. The early standards largely referred to the plate count, since at the time of their establishment it was the common method employed. More recently the increased use of the microscopic count has made necessary the formulation of requirements based on it; this has been the case with certain standards used by regulatory agencies and also with standards developed by purchasers of milk.

Bacterial standards are only a part of the requirements covering the production of milk, and laws and regulations dealing with milk supplies include many other requirements that are involved in an adequate milk control program.

In general, regulatory officials and purchasers of milk accompany their attempts to enforce bacterial standards with campaigns of education, and efforts are made to help milk producers locate the causes of high bacterial counts and correct them. Rejections of milk may be necessary when the producer is persistently careless or irresponsible. Bacteriological examinations of milk constitute a very severe test and an occasional high count may occur with milk supplied by the best producers.

Interpretation of routine counts. It should be recognized that there are limitations in both plate counts and microscopic counts of the organisms in milk so that results obtained with either method represent estimates rather than actual numbers of bacteria. Proper interpretation of the results on either raw or pasteurized milk gives significant information that is very useful to regulatory officials and milk distributors in the supervision of milk supplies; it aids in detecting unsatisfactory production and handling methods on the farms and in the plants and in establishing the safety of a supply.

Results of bacterial counts made by regulatory officials should be confidential information between such officials and the persons involved in producing or handling the milk.[21] They should not be published for interpretation by consumers who do not appreciate the limitations of the procedures employed to obtain the counts and who may attach an unjustified significance to differences in counts. Such publication sometimes has resulted in uninformed persons changing milk distributors because the milk of the new distributor was reported to have an insignificantly lower count than that of the old.

Farm and plant inspection. Inspection of the farms producing the milk is a definite part of a milk control program. There is a difference of opinion as to the relative importance of such inspection and examination of the milk, including bacterial counts, as it is delivered to the milk plant or directly to the consumer. Some milk sanitarians contend that farm inspection is of primary importance, since low bacterial counts are possible with infected cows, insanitary privies, contaminated water supplies, etc.; moreover, farm inspection has educational features for the milk producers that naturally result in improved methods and equipment on the farms. The opposite view is that when the milk reaches the plant or consumer with a satisfactory bacterial count and good qualities generally, conditions of production are satisfactory, except for such factors as can be determined by an occasional farm inspection. Proper balance between the two types of supervision is advisable and this is dependent on the general qualities of the supply and on the effort that has been put into its improvement. It can be determined better by the supervising agency than by general requirements intended to cover milk supplies produced under a variety of conditions. The object of milk control work is a safe milk supply, not the apparent compliance with a set of production requirements, and a personnel having an appreciation of the factors involved in producing milk of satisfactory quality is the best means of obtaining such a supply.

In some areas extensive farm inspection is carried out by milk distributors in an attempt to improve the quality of the milk they receive. When done by well-trained persons, such inspection often is very effective in quickly eliminating undesirable methods on the farms.

Milk plant inspections also are necessary in adequately supervising a milk supply. The proper balance between such inspections and examination of the milk ready for the consumer likewise is dependent on various factors and can best be determined by the supervising agency. Frequent plant inspections by persons connected with the operating organization also are advisable.

Examination of animals producing milk. Because of the importance of healthy, well-nourished animals in the production of an adequate milk supply, veterinary inspection of the cows is very important. Through use of physical examinations and various tests, diseased animals are eliminated from the herds and the possibility of pathogenic organisms being present in the milk is reduced. In the development of adequate milk supplies, veterinarians are employed by milk producers, milk distributors, and control officials.

Local or state supervision. Many points are involved in a decision as to whether milk supervision should be on a local or state basis and it may be that the decision should differ with different states because of variations in conditions.[22] Supervision on a local basis has resulted in lack of uniformity in requirements throughout a producing area because of variations in the regulations of different cities. Apparently, producers sometimes have changed their markets in order to operate under less rigid requirements. Milk distributors selling in two or more near-by cities or states may be greatly inconvenienced by unessential differences in regulations. On the other hand, efficient local supervisors have developed some excellent milk supplies because of a special interest in milk production and distribution and a detailed understanding of conditions in the immediate area. Through good leadership they can improve a supply until it is much above any level that is likely to be attained over an extended area. In an emergency they can act very quickly.

State control provides some protection for municipalities that otherwise might not have it, through lack of interest. It can exclude from regular milk supplies a product of inferior quality, instead of permitting it to be sold in some area other than the one in which it was found unsatisfactory. It tends to make regulations uniform over a large area and to favor reciprocal arrangements with other states. Uniform regulations that are generally accepted as satisfactory will simplify the sale of milk in distant markets where supplies are inadequate, although there is a definite tendency to be less critical when supplies are short.

The important point is to have a sound milk supervision program and a trained personnel to carry it out. Requirements should include only those that are significant from the standpoint of milk production and definitely should avoid those which are controversial and which probably are of no importance from the standpoint of milk quality.

With a sound program and an efficient personnel, extent of the inspection area is of no great importance. If the supervision is to be on a local basis, general uniformity over a producing area can be obtained by uniform regulations that are established and co-ordinated by the state. Uniform requirements [23] in milk supervision over a wide area are very desirable and would be a great stimulus to adequate milk control.

RESEARCH COUNTS

Both plate and microscopic counts are widely used in research on dairy products, the one that is adopted depending primarily on the main objective. Often the two methods are used synchronously since microscopic counts aid in determining whether all the morphologic types of organisms that are numerous in the material under investigation are developing on the plates. In some instances bacteria that were abundant in a product have been missed entirely because they failed to form colonies under the conditions provided; this would not have occurred if the results had been checked microscopically.

Counts made by the microscopic method may also need to be verified. Whitehead [24] noted that when organisms grown for 24 hr on an agar medium were added to milk and microscopic counts made, results with certain species were satisfactory, whereas with other species it was rarely possible to detect the added organisms. It appeared that some species did not acquire staining properties sufficient to render them visible until they had begun to multiply in the milk.

In employing the plate method in investigational work it is usually desirable to have the counts as accurate as possible and, accordingly, special media and incubation conditions commonly are used. When some particular group of organisms is being studied, a medium and an incubation temperature that are especially favorable can be adopted.

Media for plate counts. The agar used for routine plate counts on milk is very satisfactory for many of the organisms present in dairy products and has been extensively used for research counts. Ordinary nutrient agar is much less satisfactory and simple modifications of it have been suggested. Sherman [25] reported that addition of lactose increased both the number and size of the colonies developing on plates poured with milk and incubated 48 hr at 37 C (98.6 F). Other investigators have noted this same effect when different sugars were added to various media.

Beef infusion [26] agar is very useful for research counts; with it, colonies often are quite large. Tomato [27] agar has been employed extensively in studying the acid-producing organisms in sour milk and cream, lactic acid cultures, butter, and various cheeses. For special purposes a medium can be prepared from the product involved; butter serum, extracts or suspensions of cheeses, etc., have been employed as bases for agar. Special media adapted to the growth or differentiation of specific organisms often are helpful, such as lactate agar for the propionic acid organisms.

In some instances it is desirable to restrain the growth of certain organisms so that species of particular interest can be counted or isolated more readily. This sometimes can be accomplished by addition of an organic acid, sodium chloride, or some comparable material.

Differential media. Media which permit detection of proteolytic, acid-forming, lipolytic, or other types of organisms are of special interest in investigations on dairy products. At one time litmus lactose gelatin was employed extensively as a differential medium, but the rapid liquefaction that occurs with certain organisms limits its usefulness.

Proteolytic and acid-forming organisms. Ayers [28] described a casein agar with which proteolytic bacteria can be detected by clearing of the casein around the colonies. With it, total counts are comparatively low unless long incubation is used. Ayers and Mudge [29] proposed various milk powder agar media that permit detection of proteolytic and acid-forming organisms through their action in digesting and precipitating the casein, respectively. They gave higher counts than nutrient agar. Zoller [30] developed a milk powder agar on which proteolytic and acid-forming organisms can be detected; sodium citrate is used to prevent excessive sedimentation of lime salts and protein. The medium gave higher counts than nutrient agar. Simple addition of 0.3 to 1.0 ml of sterile skim milk to a plate before pouring with various media permits the detection of proteolytic organisms and, when sufficient sugar is present, of acid-forming organisms also. If the medium is in relatively large volumes instead of tubes, the required amount of milk can be added to it just before the plates are poured. Safford and Stark [31] used an agar medium containing skim milk and glucose, together with peptone and beef extract, on which acid-producing and proteolytic bacteria were differentiated. With this medium the counts on 618 samples of pasteurized milk were two to four times as high as on nutrient agar, whereas the counts on 137 samples of raw milk were only slightly higher; the colonies also were larger. The medium supported growth of bacteria responsible for mastitis in cows.

For differentiation of acid-forming and non-acid-forming bacteria, Wade *et al.*[32] used a medium that included, per 100 ml, 0.4 per cent calcium carbonate and 0.2 ml of 1.6 per cent alcoholic solution of bromcresol purple; it was found expedient to add the carbonate from a water suspension by means of a pipette. The carbonate delays diffusion through the agar of the acid produced by acid-forming colonies and, as a result, the indicator changes color only in the zone surrounding the colony. Without the carbonate, rapid diffusion of acid makes it difficult to distinguish between acid-forming and non-acid-forming colonies.

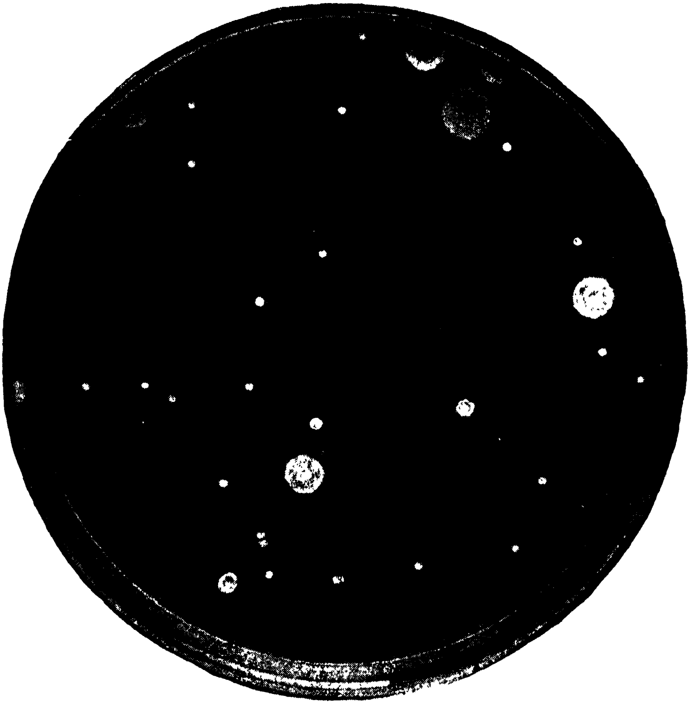

FIG. 1. Agar plate poured with added fat emulsion. Around most of the colonies the finely divided fat globules are opaque, indicating lipolysis.

Lipolytic organisms. Lipolytic organisms can be detected on plates in various ways [33] if fat is dispersed in the medium at the time of pouring; observations commonly are made with low magnification. A suitable fat distribution is easily obtained with a fat emulsion prepared as follows: Three ml of fat (cottonseed oil, butter fat, or other fat) and 0.5 gm of agar are sterilized in 100 ml of water and, after cooling, the fat is dispersed by vigorous agitation. The mixture should be rewarmed before the agitation if butter fat, or other fat that is not liquid at ordinary temperatures, is used. About 0.5 ml of the emulsion is added to each plate just before it is poured with beef infusion agar or other medium.

Fat globules around colonies of actively lipolytic organisms have an opaque appearance that is conspicuous with a low melting point fat, such as cottonseed oil. When butter fat is used it should be melted by holding the plates at 37 C (98.6 F) just before the observations are made. Weakly lipolytic organisms often are difficult to detect with this method.

Partly hydrolyzed fat is readily differentiated by flooding the plates for 30 min with 1 to 1,500 aqueous nile blue sulfate solution and then rinsing with water; if the rinsed plates are held several hours at about 45 C (113 F), the staining is intensified. Globules of unhydrolyzed fat are pink and globules containing partly hydrolyzed fat are blue so that around colonies of actively lipolytic organisms a zone of blue globules is evident. With weakly lipolytic organisms the blue globules may be found only under the colonies. A saturated solution of copper sulfate also can be used to flood the plates; [34] with this reagent the hydrolyzed fat is stained bluish-green, owing to formation of a copper soap, but the differentiation is not as sharp as with nile blue sulfate. With either reagent washing of the organisms over the surface of the plate is a serious objection when colonies are to be picked.

A sterile aqueous solution of nile blue sulfate can be added to the medium and fat emulsion at the time of pouring the plates;[33] the usual concentration is 1 part of dye to 10,000 or 15,000 parts of medium. With this procedure there is an unusually good differentiation of partly hydrolyzed and unhydrolyzed fat, but the nile blue sulfate is toxic for various organisms, especially streptococci and micrococci, so that both the total and lipolytic counts are low.

In order to impart sufficient color to the fat droplets without making the medium toxic for organisms, Knaysi [35] suggested shaking the neutral fat to be tested with a saturated aqueous solution of sulfate or chloride of nile blue, in the proportion of 1 ml of the dye solution to 10 ml of fat. The deep red fat is separated from the blue solution with a separatory funnel, washed several times with distilled water, and sterilized in the autoclave; 1 ml of the red fat then can be shaken with 100 ml of the agar medium and used for plating. The stock of sterile red fat can be stored in the icebox for several weeks. Knaysi also suggested using almost exclusively the oxazine base of nile blue for the detection of fat hydrolysis by liberating the base, from a solution of its salt, with sodium hydroxide, dissolving the washed precipitate in the fat, and shaking the fat into the medium. The base was liberated with normal sodium hydroxide added drop by drop until precipitation was complete, and the precipitate filtered off and washed with distilled water adjusted to a *p*H of about 7.5. The precipitate can be dried and stored or dissolved in fat and the fat sterilized. The fat should be nearly saturated with the base to get the maximum contrast. With this technic it was found that not only nile blue and some related dyes could be used but that a number of common basic dyes (methylene blue, neutral red, malachite green) could be employed to detect lipolysis.

For the detection of lipolytic bacteria, Starr [36] suggested an agar containing cottonseed oil emulsion and spirit blue as the indicator. Sterile plates of the medium are pale lavender in color. Colonies of lipolytic organisms are recognized by development of a permanent deep-blue color beneath and surrounding them. There was no evidence of inhibition of growth or lipolysis because of the indicator.

If tripropionin or tributyrin [37] is dispersed in an agar medium as fine globules there is a disappearance of the globules around colonies of lipolytic organisms because the products of hydrolysis are completely soluble. However, these simple triglycerides are more easily hydrolyzed than natural fats and, therefore, the results may be misleading.

Both the proteolytic and lipolytic organisms can be differentiated on plates poured with agar containing milk and finely divided fat. After incubation the proteolytic colonies are detected on the basis of clearing of the casein around them, whereas the lipolytic colonies are detected on the basis of the opaque condition produced in the fat globules or the plates can be flooded with nile blue sulfate solution in the usual way. Many of the actively lipolytic organisms also are proteolytic.

Oxidase-producing organisms. Castell and Garrard [38] detected oxidase-producing organisms by flooding plates in which colonies had grown with a 0.5 per cent aqueous solution of either *p*-aminodimethylaniline hydrochloride or tetramethyl-*p*-phenylenediamine hydrochloride. As soon as the agar surface was completely covered with the dye solution, it was poured off and the plates left with their covers removed. Fresh dye solutions were almost colorless. After contact with the dyes, oxidase-forming colonies very shortly assumed a deep color. With the tetramethyl compound the color was purple, and with the dimethyl compound it was rose red. The tetramethyl compound is more sensitive and acts more quickly. With it, many strongly oxidizing colonies take on a color almost immediately. Others, especially those submerged in the medium, react more slowly. With the dye, final observations are made at not more than 10 min after the plates have been flooded. With some organisms further action of the colony which produced the color may reduce it to a colorless form again. In other words, the reaction of the tetramethyl dye, although more sensitive, is not always permanent. The dimethyl compound is slightly less sensitive and reacts more slowly; instead of decolorizing on standing, further oxidation changes the rose-red color to a permanent black.

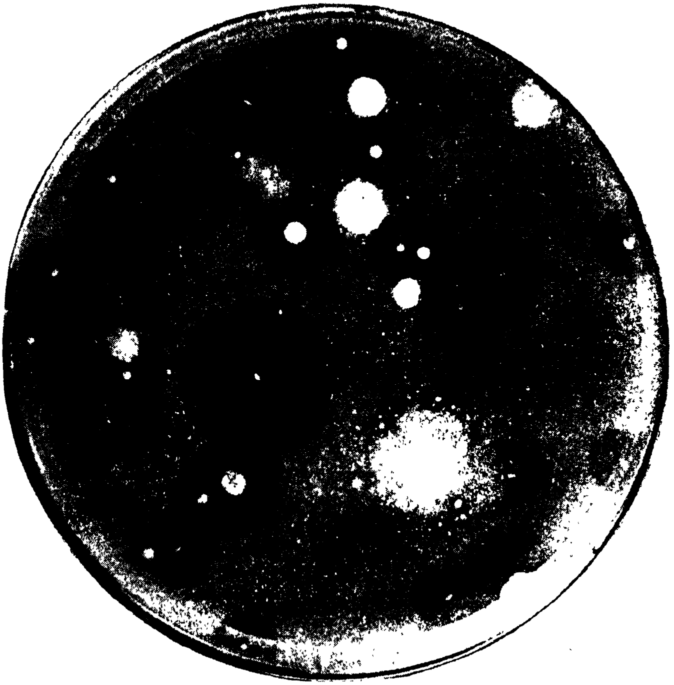

FIG. 2. Agar plate poured with added milk and fat emulsion. Many of the colonies are proteolytic, as shown by clearing of the casein, and around certain colonies the fat globules have become opaque because of lipolysis.

Incubation conditions. Incubation at 21.1 or 30 C (70 or 86 F) for several days commonly gives higher counts on dairy products than incubation at 37 C (98.6 F) or 35 C (95 F) for 48 hr, and can be used in research since it is not necessary that results be obtained quickly. Among the organisms present in milk and its derivatives, species so definitely psychrophilic that they fail to grow at 37 C occasionally are encountered. With an incubation period of only 48 hr, Pederson and Yale[39] found that 32 C (89.6 F) gave higher counts than 37 C when nutrient agar was used to plate samples of milk and ice cream. At 32 C there also was less error in counts due to the temperature variations in the incubator than at 37 C. Later, Yale and Pederson[40] reported that with 48-hr incubation and standard agar the maximum number of colonies developed at temperatures slightly below 30 C with raw milk and at temperatures slightly above 31 C (87.8 F) with pasteurized milk.

When thermophilic organisms are of importance plates are incubated at relatively high temperatures, such as 50 or 55 C (122 or 131 F). Occasionally, it is desirable to incubate plates at 10 C (50 F), or lower.

SPECIAL METHODS OF COUNTING BACTERIA

Although most of the bacterial counts on dairy products have been made with either the plate or the microscopic method, other procedures that are useful under special conditions have been suggested.

Burri smear culture technic. Burri[41] suggested a method of making counts of the organisms in milk by smearing it on the surface of an agar slope with a standardized loop and counting the colonies that develop on incubation. His general observations on the procedure are: A loop adjusted to deliver 1 cu mm, as determined by weighing the milk, is very satisfactory. However, any ordinary loop can be used; it can be calibrated by determining the number of bacteria in milk with the plate method and comparing this with the number of colonies developing when a loop of the milk is used to inoculate a plate. Relationship of the volume of milk delivered by the loop to 1 ml should be verified by repeated trials. Since as many as 100 colonies can be counted on a slope with a magnifying lens, a 1-cu-mm loop of undiluted milk gives counts from 1,000 to 100,000 per milliliter. By diluting the milk 1 to 100, counts from 100,000 to 10,000,000 can be obtained; counts made with water dilutions should be multiplied by 1.4 because of the change in surface tension that influences the amount of material delivered. Various media and incubation conditions can be used with the method. The agar slopes should be dried since water of condensation in the tubes is objectionable. With low-count milk more than one loop can be used per slope. The procedure is simple, economical, tends to avoid air contamination, and permits a good differentiation of the organisms since the colonies are all surface colonies; the slopes can be kept for extended periods if additional observations are desired. The method gives results that are somewhat higher than those obtained with the plate method, which is explained by the optimum conditions of development offered at the surface of the agar.

Cunningham and Andrews[42] compared the accuracy of the plate and Burri smear culture methods and found that the percentage error with the Burri method was markedly higher than with the plate method. They consider the inaccuracy of the Burri method is due partly to errors in loop measurements and partly to inferiority of the conditions for growth on slopes compared to those in plates.

Oval tube technic. Myers and Pence[43] suggested a loop measurement, oval tube technic for making laboratory pasteurization tests on individual producer samples after the technic was found to give reliable counts on the heated milk. A 5-ml sample of milk is laboratory pasteurized and then vigorously shaken 50 times to compensate for the breaking up of clumps in the usual plating. A loopful (0.001 or 0.01 ml) of the pasteurized milk is transferred to an oval culture tube containing about 4 ml of standard agar that has been sterilized and cooled to 45 C (113 F). The loop is moved back and forth through the agar to remove all the milk. The mixture of agar and milk then is shaken by swinging the tube back and forth through a small arc for about 5 sec. The tube is placed on a table with the stoppered end resting on a strip of wood or metal 1/8 in. thick to permit the agar to flow to a point 2.5 to 3 in. from the bottom of the tube. When the agar has solidified, the tube is placed in a special wire rack in a horizontal position but inverted. After incubation, the colonies are counted by placing the tube over a well-lighted colony counter. Myers and Pence prefer oval tubes without an indent, since they are easy to inoculate and clean. A tube can be plugged readily by wrapping the cotton around a strip of folded aluminum foil which then is folded in the middle and pushed into the oval tube.

The oval tube technic can be applied to the examination of dairy products in a variety of ways, particularly when the approximate bacterial count of the material is known so that suitable numbers of colonies can be obtained without preparing an excessive number of tubes; the medium and incubation conditions can be varied as desired. Compared to the usual plating procedure, the method saves time, materials, incubator space, etc. It also can be applied to the counting of yeasts and molds in dairy products.

Roll tube technic. The original roll tube technic involved introducing the test material into gelatin contained in an ordinary test tube, closing the tube with a cotton stopper covered by a rubber cap, and rotating it by hand under a stream of cold water until the gelatin congealed on the walls. The procedure has been refined by introducing various improvements, including motor-driven rotating devices, special tubes, agar media, etc.

Prouty *et al.*[44] compared the roll tube and the standard plate methods of making counts on milk samples. The roll tube method gave a slightly lower count than the plate method but the difference did not justify its rejection. The method possesses the usual advantages of any procedure in which tubes are used instead of plates but requires special motor-driven equipment for rotating the tubes, and a fan or cold room may be needed to congeal the agar in warm weather, even when 2 per cent, or more, agar is employed. Prouty *et al.* found the tubes more difficult to count than plates, although a special counting lens was available. Thomas *et al.*[45] reported that, on the whole, counts by the roll tube technic tended to be higher than plate counts; they suggested that rolling of the tubes results in a more thorough mixing of the inoculum and medium which may break up more bacterial clumps.

Microscopic colony count. Frost[46] devised a microscopic colony count, or little plate method, for counting bacteria in milk. The general procedure is as follows: A small amount of milk, commonly 0.05 ml, is mixed with liquefied agar at about 45 C (113 F) on a sterile glass slide, and the mixture spread over a definite area, such as 4 sq cm. After hardening, the preparation is held in a moist chamber for some hours to permit the organisms to develop colonies, and then quickly dried. Finally, the slide is stained with methylene blue or thionine, the colonies in a number of fields counted under a microscope, and the organisms per milliliter of the milk then calculated; the magnification used can vary widely. Milk that is expected to give a high count is diluted before the little plates are prepared.

The advantages of the method as listed by Frost[47] are:

1. It is rapid, requiring not more than 7 or 8 hr.
2. It is reliable in that failure due to spreaders or improper dilutions is unlikely.
3. A relatively large amount of milk is studied, usually 0.05 ml.
4. A permanent record is provided.
5. It requires only a small amount of glassware and medium.
6. The little plates are quickly made.
7. It is simpler than the plate method.

Hatfield and Park[48] reported favorably on the little plate method.

With it, certified milk showed the smallest average variation from the standard plate counts. The larger variations with pasteurized milk and raw milk to be pasteurized were about equally divided between counts higher and counts lower than the plate results. Bryan *et al.*[49] found that incubation of Frost little plates in a moist chamber at 37 C (98.6 F) for 4 hr yielded bacterial colony counts on milk and cream comparable to standard plate counts.

Field procedures. When it is necessary to make cultures where facilities are very limited, bottles or flasks with cotton stoppers or screw caps can be employed; a type of bottle with at least one flat side on which it can be rested to give a layer of agar of uniform thickness is advisable. The containers are sterilized with agar in them and, when needed, the agar is melted in boiling water and cooled for inoculation. The inoculum is added, usually with a loop although pipettes and water blanks may be employed, and then the agar is solidified. After incubation, the colonies are counted.

Bell[50] devised a special rectangular culture flask of heat-resistant glass for use in milk receiving stations, etc. It has an offset, round neck so that 10 ml of sterile agar and 0.001 ml of milk can be mixed without wetting the cotton stopper. The flask, containing sterile agar, is taken to a receiving station or plant and the agar melted by placing the flask in boiling water. After cooling, it is inoculated with 0.001 ml of milk, using a loop, and the agar and milk mixed and solidified. After incubation, the colonies are counted.

As an emergency method for estimating bacterial populations, Hasson[51] suggested the flooding of slabs of solid medium in bottles, with a suspension of bacteria in some material such as milk and then draining, incubating, and counting the colonies that develop. By controlling the factors influencing the counts, reproducible results were obtained. Results can be correlated with actual plate counts and a conversion factor established.

COLLECTION AND CARE OF SAMPLES

Samples of milk, cream, and other materials that are to be examined bacteriologically must be collected, cooled, and held under conditions that avoid contamination and prevent growth of the organisms present. Standard procedures for the collection and handling of routine samples of the usual types have been developed[1] and should be followed.

Collection of samples for research purposes, or for the control of plant processes, sometimes presents difficulties. Lines for carrying milk or other materials can be tapped and outlets attached but when samples are obtained in this way it is essential that the outlets be well steamed and well flushed with the product before the sample is collected.

In sampling large volumes of milk, cream, etc., in tanks, thorough agitation is very important, particularly when the material is made up of small lots from many sources and there is no agitation during the collection. If the level of the product in the tank is beyond the reach of sampling tubes, etc., it can be sampled by lowering a sterile bottle into it. A convenient procedure is to sterilize a bottle and attached string in a container, such as a glass jar, that can be closed with a lid or parchment paper; when needed, the container is opened, the stopper is removed from the bottle, and the bottle is lowered into the product until it is sufficiently filled, when it is raised and stoppered, all with precautions against contamination.

Proper collection and holding of samples are essential in all bacteriological work. Without representative samples, any results that are obtained will be open to question. Undoubtedly, much effort has been expended on samples that could not give reliable results because the collection and/or care was faulty. Bacteriological examination requires much time and such time is wasted unless the samples used are properly collected and held.

LINE TESTS

Line tests, in which a series of samples is taken during a processing operation, are widely used in connection with bacteriological control of dairy products and in research on them.

If milk supplied by a certain plant has a high bacterial count, a line test may be run in an attempt to locate the irregularity. The usual procedure is to take a series of samples, beginning with the raw milk, at various points in the processing; the points often are determined by the opportunities for the collection of special samples. If an undesirable microbiological condition is found in the product of a butter plant, a series of samples can be taken, beginning with the raw cream and including freshly pasteurized cream in the storage vat, cream after holding in the vat with or without culture, cream in the churn, cream after the churn has been operated briefly, buttermilk and granules as soon as granules have formed, wash water, washed butter, worked butter, butter as it comes from the churn, and butter after various holding periods. Results on a series of samples commonly show the source of excessive numbers or objectionable types of organisms.

A series of samples also can be taken in attempting to determine the changes in the micro-organisms during a normal operation, such as processing of sweetened condensed milk, production of whole milk powder, or manufacture and curing of a cheese.

Line tests are extremely useful in both control and research work. It is essential that the samples be collected and held under the best possible conditions. Without proper samples, the results are open to question and they may even be misleading.

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Chapter 2

Tests for the General Quality of Raw Milk

In addition to enumeration of the contained bacteria on a total or differential basis, various tests are used in determining the general quality of raw milk. Some of these involve the detection of the products of growth of organisms; some involve the rate at which changes are produced by the contained organisms and, in general, are related roughly to the numbers present; some involve the presence of certain types of organisms, including types objectionable in milk processing; and some are intended to detect udder infections. Often the tests are simple so that they can be carried out by persons without special training and can be applied to many samples in a relatively short period. Certain of the tests are applicable to the examination of cream.

TESTS FOR PRODUCTS OF THE GROWTH OF ORGANISMS

Under the usual conditions of producing and holding raw milk, extended growth of organisms results in the formation of products that give an abnormal odor and flavor and, eventually, a definite increase in acid, and also an instability that is detected on mixing with alcohol under standard conditions. The presence of these products constitutes the basis of some of the tests used with milk.

EXAMINATION FOR ODOR AND FLAVOR

Milk received at dairy plants is examined commonly for odor and sometimes for flavor. Plant employees may hesitate to taste the raw product because of the possibility of contracting a milk-borne disease. Many flavor defects are readily recognized by the odor alone; perhaps the outstanding exceptions are bitterness and saltiness and, ordinarily, these are of relatively little importance in milk. The odor when the cover of a can of milk is first raised is especially valuable in indicating a slight defect.

Abnormal odors in milk may be due to any one of a number of causes, such as absorption of odors, materials consumed by the cows, condition of the cows, chemical action, addition of foreign materials, and action of micro-organisms. Those due to organisms are of various types but an odor suggestive of acid, often modified by some other odor, is the most common. The experienced grader of milk and cream learns to recognize the causes of abnormal odors and, with those due to organisms, often can identify the particular species or group involved. Recognizing the cause of a defect is especially helpful in advising the producer as to methods of correction.

TITRATION FOR INCREASED ACIDITY

Milk sometimes is titrated for the purpose of detecting increases in acid. Because the initial acidities of different lots of milk vary so widely, such tests can be only very crude; they are much less sensitive than organoleptic examination by an experienced person which commonly detects very small changes in acid. Definite increases in titrable acid due to growth of bacteria occur only when large numbers are present. The chief value of titrations is in connection with establishment of standards that exclude from certain uses lots of milk with relatively high acidities.

Variations in the initial acidities of lots of milk depend in part on the composition. Some lots of milk with high total solids contents may have acidities higher than any standard likely to be established, even when there has been no opportunity for growth of organisms, whereas other lots may have such low total solids contents that even with considerable increases in acid the acidities are below any such standard. Mixing of milk from a number of animals eliminates some of the variations in initial acidities but they still are evident in herd milk, especially when different breeds are involved. The acidity of cream is lower than that of the milk from which it came, the extent being related to the fat content, but often the same standards are applied to the two products.

Titrations have been of special value in the grading of cream for butter. The butter industry normally uses some cream in which rather extensive growth of organisms has occurred. In such cream most odors and flavors due to organisms are objectionable and, commonly, they are not as conspicuous with an acidity of 0.2 per cent, or less, as with a higher one. Cream which has developed considerable acid may be placed in more than one grade. Grading of cream on the basis of acid can be carried out rapidly by mixing cream and standard alkali, containing indicator, in definite proportions and then noting whether the reaction is acid or alkaline.

ALCOHOL TEST

The alcohol test consists of mixing equal parts of milk and ethyl alcohol of a definite percentage in a tube and noting whether coagulation occurs. With normal milk the mixture breaks clear from the wall of the tube; with milk showing certain abnormalities the wall is clouded with fine particles of curd and the mixture shows a precipitate of milk constituents. The alcohol employed usually has varied from 68 to 75 per cent by volume. It can be prepared by diluting high-grade alcohol with distilled water. The diluted alcohol should be tested for acid since acetic acid sometimes is present and may influence the test; alcohol containing acid should be distilled in the presence of sodium hydroxide. The test usually is made at room temperature, using 2 ml of milk.

Conditions causing coagulation. From the data available it is evident that products of the growth of organisms may be responsible for coagulation in the alcohol test, and also that certain other factors may be involved.

One of the causes of a positive test is the presence of developed acid in milk. However, Dahlberg and Garner[1] found no direct relationship between coagulation of milk with alcohol and its titrable acidity, although milk high in acid gave coagulation in the majority of cases. Development of a bacterial rennin in milk may be a factor in the test; Sommer and Binney[2] noted that the coagulating enzyme produced by 856,000 rennin-forming organisms per milliliter was sufficient to give a positive result. Ayers and Johnson[3] found no definite relationship between numbers of bacteria in milk and coagulation with alcohol. Of 177 samples of raw milk studied, 19 were positive with 68 per cent alcohol and, of these, 8 (42.1%) contained less than 500,000 bacteria per milliliter, whereas 11 (57.9%) contained more than 500,000; among the negative samples, a considerable number contained more than 500,000 bacteria, the maximum being 20,200,000.

Colostrum and milk from cows advanced in lactation usually are positive with the alcohol test and some milk from abnormal udders also is positive. This suggests that certain abnormalities in the composition of milk result in coagulation when the milk is mixed with alcohol. If milk that coagulates with alcohol is mixed with normal milk, the mixture may not coagulate; Ayers and Johnson[3] found that a mixture of colostrum and normal milk would not give a positive test unless a very large percentage of the mixture was colostrum.

Sommer and Binney[2] investigated the influence of the salt balance of milk on the alcohol test and noted that a slight increase in magnesium or calcium content gave a positive result, whereas an increase in potassium, sodium, chloride, citrate, or phosphate did not. The effect of magnesium and calcium is counteracted by citrates and phosphates so that a positive test depends mainly on the relative amounts of the four constituents. The investigators found that a high calcium content in a ration fed to cows may cause a positive alcohol test in the milk.

Weimar[4] reported instances in which a positive alcohol test was due to feeding silage of poor quality. A study of samples of milk from individual animals, only a part of which was positive, indicated that bacterial content and acidity were of no importance and suggested the influence of the silage, since the cows giving milk that was negative had refused the silage. Experimental feeding with three animals showed that the effect of alcohol on the milk could be changed by varying the feed.

Modifications of the test. Methods of titrating with alcohol have been devised, the end point being considered the first appearance of coagulation in the milk. In general, these have no advantage over the method of mixing equal volumes of alcohol and milk.

Chalmers[5] modified the alcohol test by subjecting the milk to a preliminary incubation of at least 9 hr at 37 C (98.6 F), and then adding 83 per cent (by volume) neutral alcohol containing 0.02 per cent methyl red. The incubation permits development of the contained organisms under standard conditions before the milk is examined for products of their growth, while the indicator gives information as to the acid present. Cooling the milk before the reagent is added appears to be important from the standpoint of the color obtained with the indicator.

Uses of the test. The alcohol test has been suggested for use with milk intended for condensing or drying. Its detection of unusual salt balances in addition to certain other abnormalities may be of importance in this connection.

TESTS INVOLVING THE RATE AT WHICH CHANGES ARE PRODUCED BY THE CONTAINED ORGANISMS

The rate at which a specific change is produced by the organisms in milk is the basis for a number of tests used to arrive at its microbiological quality. Normally, such a change is related roughly to the numbers of organisms present but also it is greatly influenced by the species of organisms.

KEEPING-QUALITY TEST

A simple keeping-quality test can be made on milk by holding samples under more or less definite conditions and, from time to time, observing them for changes. The portions can be held in test tubes or small bottles that have been sterilized or thoroughly steamed and should be transferred under conditions that prevent contamination. A constant temperature is preferable for holding but it is not necessary and the samples in a series can be compared at any temperature reasonably satisfactory for growth of organisms. The period required for coagulation is a convenient basis for comparison, although the acidity at which coagulation of milk occurs varies somewhat with different lots; it is a more satisfactory basis than the period required for development of an acid flavor because of the difficulty of judging when an acid flavor is present.

The character of the curd formed gives information of importance concerning the organisms in the milk. Gas-producing species, types decomposing casein rapidly, and types producing objectionable odors often can be detected by examination of the curdled material. Such information is of special significance with milk to be made into certain cheeses, although the influence of temperature and rapid acid production may tend to control certain undesirable fermentations during cheese manufacture and curing.

KEEPING QUALITY IN THE PRESENCE OF AN INDICATOR

Baker and Van Slyke[6] proposed a method of determining the keeping quality of milk in the presence of bromcresol purple that permits recognition of slight changes in acid. Three ml of milk and 1 drop of a saturated aqueous solution of the indicator are mixed in a tube, with precautions against contamination, and incubated at a definite temperature, usually 18 to 20 C (64.4 to 68 F). At intervals the milk is examined for changes in color. As the acid increases, the color varies from the grayish blue in normal milk to a final clear yellow. Changes involving digestion, gas formation, etc., also are evident after prolonged incubation. Baker and Van Slyke[7] noted that addition of bromcresol purple to milk makes possible the preliminary detection of abnormal milk. The color is made lighter than normal by acids, acid salts, formaldehyde, and heating above the usual pasteurization temperature, whereas it becomes darker with milk from diseased udders, watered milk, skim milk, and milk containing alkali or alkaline salts.[illegible]

CHANGES PRODUCED IN *p*H OF BOUILLON BY ORGANISMS IN ADDED MILK

Cooledge and Wyant[8] suggested a method of determining the keeping quality of milk by a colorimetric *p*H test, using 0.1 ml of the milk in bouillon containing an indicator. The general procedure is as follows:[9] One bouillon cube, 1 per cent peptone, and 0.5 per cent sodium chloride are added to each liter of water and, after heating and filtering, 80 ml of a 0.04 per cent aqueous solution of bromthymol blue is added. The broth is cooled to 20 C (68 F) and the *p*H adjusted to 6.8. It then is placed in 100- or 200-ml flasks and sterilized. When a flask of the bouillon is to be used, 10 ml is transferred to a 1.8- by 16-cm sterile test tube with a sterile pipette and compared with a standard having a *p*H of 7.0; if incorrect, the broth in the flask is adjusted to *p*H 7.0. The broth is transferred to sterile 1.8- by 16-cm test tubes, 10 ml in each. Then 0.1 ml of each milk sample is added to a tube of the broth, using a 9-ml blank, and the tubes are incubated at 37 C (98.6 F). Hourly observations are made to detect the first color change due to development of acid. As soon as pronounced changes are noted, hourly readings are made by comparing the tubes with standards of known *p*H values. The broth must be cooled to 20 C before readings are made. The method[8] makes it possible to detect the poorer samples of milk in 1 hr, whereas the best samples can be given their proper grade in 8 hr; the poorer the keeping quality of the milk, the sooner results are obtained.

METHYLENE BLUE TEST

The methylene blue reduction test involves the determination of the period required for disappearance of the color when a specified methylene blue solution is added to milk under controlled conditions. At one time an aqueous solution of a saturated alcoholic solution of methylene blue was used to obtain the desired dye concentration. Later, tablets containing methylene blue chloride were employed. In 1935, Thornton and Sandin[10] recommended that methylene blue thiocyanate be substituted for the chloride because of the reproducibility of the former dye, and it is now generally used.

A methylene blue test on a sample of milk measures one type of bacterial activity in it. In general, the more rapid the disappearance of the color, the higher the numbers of bacteria. However, various factors influence this relationship and there are numerous exceptions to it. With some samples of milk, reduction is more rapid than would be expected on the basis of plate or microscopic counts, whereas with others it is less rapid. At one time the more important causes of the irregularities were (1) different reducing powers of the various bacterial species, and (2) concentration of organisms in the cream layer by the rising fat globules; the latter now has been largely overcome by inversion of the tubes during the test.

It is essential that the test be carried out according to the standardized procedure [11] if irregularities in the results are to be kept to a minimum. Black [12] found that deviations from the requirements of standard methods were rather common.

Variations in reducing powers of different organisms. Different species of organisms vary a great deal in their reducing powers. *S. lactis* reduces methylene blue rapidly; it is important in the reduction of poor-quality milk because of its reducing ability and also because of the numbers present. Bendixen and Ellington [13] found that proteolytic, alkali-forming, inert, and high lactic-acid-producing organisms reduce methylene blue in milk and cream as effectively as ordinary lactic acid bacteria; the reducing powers of different organisms and of the same organisms under different conditions appeared to vary. Working with high-quality raw milk (average plate count about 8,000 per milliliter), Davis and Lines [14] found that proteolytic bacteria are good oxygen absorbers and play an important part in reduction; acid-coagulating, alkali-forming, and inert types had no special effect on reduction rates, unless present in unusually large numbers; and acid non-coagulating types were poor reducers. The authors reported an associative action between bacteria which usually overshadowed individual variations in reducing power. According to Hobbs,[15] the most rapidly reducing organisms belong to the coliform group; following them in decreasing order are *S. lactis* and some of the fecal streptococci, *Staphylococcus* species (*Staph. aureus, Staph. albus, Staph. citreus*), certain micrococci, certain hemolytic streptococci, some strains of *Streptococcus agalactiae,* and aerobic spore formers.

Effect of inversion of test portions. Early observations indicated that occasional inversion of tubes of milk containing methylene blue shortened the reduction period because it tended to keep the bacteria distributed through the milk instead of permitting their concentration in the cream layer. Inclusion of the inversion of the tubes as a part of the methylene blue test was first accepted in England. Thornton [16] noted that without inversion replicate samples of high-quality milk frequently exhibited serious variations in reduction periods and that the variations tended to increase in frequency and magnitude as the reduction period increased; with shaking of the tubes during incubation the variations practically disappeared and the reduction periods were shortened. Frayer[17] reported that inversion of the tubes resulted in shortening the reduction periods and rendered the tests more significant. With periodic inversion of the tubes during incubation, Abele[18] found a higher, although still imperfect, agreement between plate counts and reduction periods and a nearly complete agreement with respect to percentages of plate counts over 200,000 per milliliter and reduction periods shorter than 6 hr.

Influence of various factors on test. Various factors influence the methylene blue test. Most of the studies along this line were carried out before inversion of the tubes was the standard procedure but they probably apply to the test with or without inversion.

Hastings *et al.*[19] found that the reduction period was prolonged as the concentration of dye was increased. Age of the methylene blue solution had little if any effect on the reduction period, solutions 2 yr old giving essentially the same results as fresh solutions. In the investigation of the influence of temperature, 28 C (82.4 F), 33 C (91.4 F), and 38 C (100.4 F) were used; with the majority of the samples the highest temperature gave the most rapid reduction, but with certain of them the reduction period at 28 C was shorter than at 38 C. The bacteria common in milk show great variations in their optimum temperatures and, since certain of these are considerably below 38 C, some samples would be expected to reduce more rapidly at lower temperatures than at 38 C.

Fay[20] studied the normal variation in the methylene blue test, making 75 to 100 determinations on each of 19 samples, and found that the maximum and the minimum reduction periods for each sample were relatively close together. He also noted that the various modifications intended to simplify the test, such as using a standard 10-ml dipper rather than a 10-ml pipette, graduated test tubes instead of a 10-ml pipette, glassware that had been boiled rather than sterilized, and methylene blue powder rather than a standard tablet, did not seriously impair the accuracy of the test. In trials involving variations in the quantity of dye, the reduction time was directly related to the amount of dye used.

Light has a definite effect on the period of reduction of methylene blue. Aikins and Fay[21] noted that light hastened reduction of methylene blue in market milk; light from a 75-watt electric lamp had an effect that differed only in degree from that observed in sunlight. The reduction of methylene blue in one sample of milk was hastened 2.5 hr by light from an electric bulb. Frayer [22] reported that light, either artificial or natural, exerted a pronounced reducing action on methylene blue in market milk. Jackson [23] found that the reduction of methylene blue in milk is catalyzed by light in the visible spectrum. Various observers suggest that the reduction of methylene blue by light is a reaction distinct from the reduction induced by bacteria. Because of the light effect, the test commonly is carried out in the dark.

Frayer [22] noted that cooling milk and holding it at 4.4 C (40 F) for 24 to 72 hr before starting the test retarded the reduction of methylene blue. He suggested that as a result of this relationship a sample having a comparatively high bacterial content may require longer for reduction than another sample of lower count that has not been cooled. Wilson [24] studied the influence of icing for 18 to 24 hr on various types of milk, both raw and pasteurized, and found the reduction period was commonly increased, although with raw tank milk it was decreased.

Jackson [23] reported that milk as it exists in the udder, or milk drawn anaerobically, reduces methylene blue almost instantaneously, whereas the same milk exposed to oxygen usually will take more than 10 hr to reduce. The oxidation-reduction potential of anaerobically drawn milk is much lower than that of the same milk exposed to oxygen.

Mechanism of reduction. At one time it was believed that decolorization of methylene blue occurs in two steps: (1) removal of dissolved oxygen by growing bacteria, and (2) reduction of the dye by the natural reducing system in the milk. Since there is no oxygen in methylene blue, reduction involves the transfer of hydrogen. Thornton and Hastings [25] suggested that it is probable many compounds can act as hydrogen donators. Later, it appeared that the reducing substances formed by the bacteria also are a factor in the reduction and that with some organisms the reducing system formed by bacteria is more important than the reducing system of the milk. Hobbs [15] reported that the most important factor in the reduction is the formation of reducing substances by the organisms themselves and that the oxygen absorption is incidental rather than essential; on this basis the methylene blue test is an index of bacterial metabolism in the milk.

Visual reduction of methylene blue occurs over a narrow oxidation-reduction potential range. Among hundreds of milk samples studied by Frayer,[26] no instances were noted in which methylene blue was completely reduced in the presence of more than a minimum of oxygen.

Numbers of bacteria present at time of reduction. The numbers of organisms present in milk at the time of reduction of methylene blue are variable. With 38 samples Thornton and Hastings[27] found plate counts from 3.5 to 45 million per milliliter with an average of 22 million. Wilson[24] noted plate counts from 3.5 to 70 million and an average of 16.9 million with raw milk, whereas the counts ranged from 7 to 204 million and averaged 103.2 million with pasteurized milk.

With milk having a low plate count originally, Davis and Lines[14] found no direct relationship between the counts before and after reduction. As the reduction period increased there was an increase in the count after reduction; their results are as follows:

Comparison of Reduction Period and Average Plate Count after Reduction

Reduction Period (Hours)	Average Plate Count per Milliliter after Reduction
0– 2	10,000,000
2– 3	17,000,000
3– 4	19,000,000
4– 5	21,000,000
5– 6	21,000,000
6– 7	23,000,000
7– 8	22,000,000
8– 9	33,000,000
9–10	37,000,000
10–11	36,000,000
Above 11	61,000,000

Value of test. The methylene blue test is a useful method of determining the general bacteriological condition of raw milk. Although certain samples with relatively large numbers of organisms reduce slowly and others with relatively small numbers reduce comparatively rapidly, the general relationship between reduction period and numbers of organisms holds in sufficient detail so that results of the test can be used to select the farms whose methods of producing and handling milk need improvement. When results are obtained with the test that appear illogical on the basis of the production methods used with the milk, direct or plate counts should be used as check tests; such check tests are advisable, occasionally, on a milk supply when the methylene blue test is being used as the regular examination procedure. Macy[28] employed the test in the grading of sweet cream, using a triple strength methylene blue. The period required for reduction was inversely proportional to the bacterial content. He considered the methylene blue test a satisfactory index of the bacterial content of sweet cream, and useful for the grading of sweet cream intended for butter.

Various studies have been made on the correlation between the methylene blue test and other methods of determining the general sanitary quality of milk. A close agreement cannot be expected because of the differences in the things measured by the various tests. The methylene blue test gives a general measure of the activity of organisms in samples of milk that is very useful for many purposes. Investigations indicate that the results of the test correlate rather closely with the keeping quality of milk.

Modifications of test. Various modifications of the methylene blue test have been suggested. Johns [29] proposed a preliminary holding at 12.8 C (55 F) for 18 hr, and the mixing of the contents of the tubes not decolorized in 6 hr during the following incubation. Both modifications shortened the reduction period. The chief advantages of the modified test are greater convenience, improved accuracy on milk of high grade, and closer correlation with keeping quality. Hiscox *et al.*[30] suggested carrying out the test at 15.5 C (59.9 F). Their results indicated a close correlation between development of an off flavor and disappearance of the color.

RESAZURIN TEST

The search for other indicators that might be used in place of methylene blue in reduction tests led to the introduction of resazurin and it has been studied extensively. Resazurin imparts to fresh milk a characteristic blue color. Reduction of the dye is shown by a gradual change in color through various shades of purple and mauve to full pink, when apparently resazurin is completely converted to resorufin; in milk this stage of reduction is not reversible. A second stage of reduction consists of conversion of resorufin to dihydroresorufin, with a color shift from pink to white; this reduction is reversible.

By adding resazurin to milk, one type of bacterial action can be measured, just as when methylene blue is added. In general, the more rapid the changes in the dye, the higher the numbers of bacteria. Various factors influence the relationship, however, and there is no close correlation between reduction of the dye and numbers of organisms.

Bases for reading test. With the resazurin test the results can be based either on (1) degree of color change after a stated incubation period, or (2) period required to reduce the dye to a given end point. Because of the possibilities with the series of colors involved in the reduction of the dye, the test has been read on a number of different bases. Certain of them are considered to be more satisfactory than others and methods for carrying them out have been standardized.[11]

Results obtained with test. Following work elsewhere, the resazurin test was introduced into the United States by Ramsdell *et al.*[31] They reported that, as used by them, the test could be completed in 1 hr and the milk classified into grades. Milk from diseased udders and physiologically abnormal milk also had a significant effect on resazurin.

Various additional investigations have confirmed the conclusions of Ramsdell *et al.:* (1) that results can be obtained more quickly with resazurin than with methylene blue, and (2) that certain samples of abnormal milk, due to physiological and pathological conditions (early lactation, advanced lactation, mastitis, etc.), are detectable with resazurin. The second point often is an advantage but is objected to by certain control officials who, when reduction tests are run, are interested primarily in the general bacteriological condition of the milk. For distinguishing changes in resazurin that are due to bacteria from those due to other causes, microscopic observations can be made.

Reading the resazurin test after 1 hr is most useful with relatively poor milk supplies. However, some milk samples of this type contain many dormant bacteria and they produce little change in 1 hr. To detect such samples the incubation can be prolonged. Johns [32] noted that the 1-hr resazurin test does not indicate the bacterial content of milk as reliably as the methylene blue test, with inversion of the tubes, and places a larger proportion of high-count milk in the highest grade.

Johns [33] reported that with incubation until the pink color is reached resazurin tests compare very favorably with the methylene blue test and the reduction period is shortened. Johns and Howson [34] found that resazurin reduces to the pink stage in approximately three-fourths the period required for methylene blue to reduce. The pink end point is of little value in detecting abnormal milk.

Influence of various factors on test. Various factors influence the test in much the same way the methylene blue test is influenced. Particularly, different species vary in their reducing powers.

Frayer [26] noted that low-count milk to which resazurin is added, when exposed to bright sunlight without metabolic activity previous to incubation, behaves as a rule as does a sample of poor milk in respect to rapidity of oxygen depletion and color changes.

Thomas and Probert [35] found that increased leucocyte counts were associated with well-defined increases in rates of resazurin reduction. Leucocyte counts under 500,000 per milliliter had little effect on reduction of resazurin or methylene blue, a count between 500,000 and

2,000,000 had an appreciable effect on resazurin but not much effect on methylene blue, and counts over 2,000,000 had a strong reducing effect on either indicator.

Value of test. The resazurin test determines, in a general way, the bacteriological condition of raw milk. There is no close correlation between results obtained with it and with plate or direct counts but it serves to detect milk supplies that need attention.

Since results can be obtained more quickly with the test than with the methylene blue test, it often is preferred when the day's work is normally completed at a definite hour. Detection of abnormal milk also may be an advantage under certain conditions.

The series of colors that should be distinguished in the resazurin test complicate it when the test is to be run by persons with relatively little training or with certain vision defects. Distinguishing between blue and white, as in the methylene blue test, is very simple.

Golding and Jorgensen [36] noted that the resazurin test agrees reasonably well with the plate count and can be worked out so that a plant using the test can obtain about the quality of milk desired.

Chilson and Collins [37] found that, as applied in their work, the resazurin test was a satisfactory and rapid criterion of both the initial quality and keeping quality of pasteurized 20 and 40 per cent cream; it could not be depended on to detect high counts in cream that had been stored at 4.4 C (40 F), or lower, for 4 or more days.

TESTS FOR CERTAIN TYPES OF ORGANISMS

The presence in milk or cream of relatively large numbers of organisms of a certain type sometimes permits of more or less definite conclusions with reference to the products, and various tests involving this principle have been proposed. The organisms considered in such tests are objectionable because they may produce an undesirable change, such as gas formation in cheese, or because they are associated with contamination from a specific source.

WISCONSIN CURD TEST

The Wisconsin curd test is used primarily in the detection of milk that is unsatisfactory for cheesemaking; it shows especially the presence of gas-forming organisms but also may give information with reference to other undesirable types.

Method of making the test. Samples of milk to be examined are collected in sterile pint jars or wide-mouth bottles, the containers being almost filled. By means of a water bath, the samples are adjusted to about 32.2 C (90 F) and 10 drops of rennet extract mixed with each. As soon as a firm curd is formed it is broken by shaking or cut into small pieces with a knife that has been sterilized or boiled. The container and curd are kept warm (32.2 C or higher) to favor growth of the bacteria. As the whey collects it is poured off; the curd occasionally is broken by shaking or cut to facilitate drainage of the whey. When a small pat of curd quite free from whey is obtained, the container is held at about 37 C (98.6 F). After 10 or 12 hr the curd is examined. If it is solid throughout, shows no sliminess at the surface, and has an agreeable odor, the milk probably was free from organisms that would be objectionable in cheesemaking, whereas gas holes in the curd, sliminess, objectionable odors, etc., show the presence of organisms that probably will cause undesirable conditions in cheese; in some instances the curd is so full of gas holes that it is spongy. Milk with a high bacterial count may give a desirable curd and another lot with a low count may give an undesirable curd so that the test is of no value in comparing samples of milk from the standpoint of their keeping qualities.

Value of test. Use of the test on milk from individual farms makes it possible to detect lots that are objectionable for cheese manufacture. Although results of the test are not available before the cheesemaking is begun, there ordinarily is a general similarity in the milk from a farm so that the farms needing improvements in the methods of milk production can be detected. The test is of less importance than formerly because extensive use of pasteurization of the milk has aided greatly in the control of objectionable fermentations in various cheeses.

ANAEROBIC SPORE TEST

Some of the spore-forming, anaerobic bacteria produce a "stormy" or gassy fermentation in milk that is more or less characteristic, and certain investigators have attempted to establish the significance of this change in milk held under conditions favorable for it. Weinzirl and Veldee [38] called attention to the possibility of testing milk for contamination with manure in this way. The procedure involved heating milk in definite amounts, such as 5, 10, and 15 ml, to 80 C (176 F) for 10 min to kill the non-spore-forming bacteria that may interfere with the fermentation, covering with sterile paraffin oil to a depth of about $\frac{1}{8}$ in., and then cooling and incubating at 37 C (98.6 F). If certain anaerobes are present gas will be formed and will force up the paraffin plug. The investigators considered that determination of the amount of milk required to give a gassy fermentation, under the conditions used, made it possible to obtain information on the extent of contamination with manure. Later, Weinzirl[39] used the following procedure: 0.5 to 1 ml of melted paraffin is placed in 1.5-cm test tubes which are plugged with cotton and sterilized, with either dry or moist heat. By means of a sterile pipette, 5 ml of the milk to be tested is placed in each of five tubes, and the tubes then are heated in an Arnold sterilizer to 80 C for 10 or 15 min. The melted paraffin rises to the surface where it hardens when the temperature is lowered. The tubes are incubated for 3 days at 37 C. If two tubes out of the five form gas the milk is considered to show excessive pollution.

In the studies of Ayers and Clemmer,[40] ten tubes of milk, each containing 20 ml, were used per sample and there was a fairly definite relationship between the results and the conditions of production; but it was noted that no reliance could be placed on one test and that tests on a number of samples were necessary for the results to have significance.

Hudson and Tanner[41] compared the anaerobic spore test with the microscopic colony count and the sediment test on more than 100 samples of milk. They concluded that the procedure cannot be accepted as a method of accurately determining manurial pollution of milk; no correlation was found between results of the test and numbers of bacteria or amounts of dirt.

The anaerobe of most importance in causing gas in heated milk held in deep layers appears to be *Clostridium sporogenes* but, undoubtedly, other species also are involved. The results of Ayers and Clemmer[40] indicate that the majority of the spores of *Cl. sporogenes* gains entrance to milk from cow manure. However, there are various other sources from which spores of anaerobes can enter milk.

TESTS FOR *ESCHERICHIA-AEROBACTER* ORGANISMS

The *Escherichia-Aerobacter,* or coliform, group of organisms includes the aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacteria which ferment lactose with formation of gas. The importance of certain of these organisms as an indication of the bacteriological condition of water supplies has led to attempts to use them in determining the quality of milk. Because of the various opportunities for the contamination of milk with the organisms and also for their growth in milk, conclusions with reference to them can be drawn only under specific conditions. Milk produced with excellent procedures ordinarily contains some of the organisms although it may be necessary to examine relatively large volumes to detect them.

The presence of coliform organisms in milk can be shown [11] by formation of gas in various liquid media on inoculation of the milk. Different volumes can be used and with a series of tubes for each volume the probable numbers of the coliform organisms can be determined. Also, various media can be used for direct plating. With either liquid or solid media, it is advisable to isolate and purify occasional cultures and check their important characters to be certain they belong to the group.

Coliform organisms gain entrance to milk from a variety of sources, including manure, dirt, utensils in poor bacteriological condition, the milker, flies, etc., and, occasionally, from infected udders. *Escherichia* organisms come largely from fecal material, whereas *Aerobacter* and intermediate organisms come primarily from non-fecal materials. Griffin and Stuart [42] noted that the distribution of 6,577 strains of coliform organisms in milk, water, soil, grains, and feces indicated that *Aerobacter* and intermediate organisms constitute the normal coliform flora of non-fecal materials, while *Escherichia* organisms are normal to feces. They suggested that occurrence of these groups outside the usual habitats is adventitious.

When once in milk, coliform organisms may multiply actively if the conditions permit. *Aerobacter* types multiply at lower temperatures than the *Escherichia* types, as would be expected from the sources of the two groups. Robinton and Genung [43] reported that coliform organisms develop much more rapidly in cream than in milk and the former apparently contains either some accelerating growth factor or a greater surface area of available food materials.

Significance. Sherman and Wing [44] studied the application of a coliform standard of 1,000 per milliliter to 220 samples of milk before and after holding, and found that about two-thirds of the initially satisfactory milk that was held at either 10 C (50 F) for 48 hr, or 15 C (59 F) for 24 hr, would have been condemned on the basis of containing more than 1,000 coliform organisms per milliliter. Of those held at 10 C for 24 hr, 13 per cent would have been condemned. The investigators concluded that the coliform test is of no especial value as an index to the sanitary conditions surrounding the production of raw milk of the usual grade; in the case of high-grade milk containing less than 10,000 total bacteria per milliliter the test may have a place as a supplementary index of quality. They noted that if all milk were required to be cooled promptly to 7.2 C (45 F) and maintained at that temperature, the test might prove of more general value.

Yale and Eglinton [45] concluded that the coliform test has only a slight value as a routine test for ordinary fresh milk because of the time required to make the observations necessary to determine which of the various causes of high colon counts is at fault; high counts usually are not due to manurial contamination but to growth on utensils and in milk. They found that growth in milk during the 2-hr interval between production on the farm and delivery to the plant increased the count from 4 to 52 per milliliter with one can of milk and from 1,300 to 6,200 with a second can.

Relatively large numbers of coliform organisms in a lot of milk definitely suggest an undesirable condition, just as do relatively large numbers of other groups of organisms or of total bacteria. An undesirable condition may involve methods of production and/or methods of holding. Commonly, the objectionable condition also is evident from total bacterial counts; in some instances an irregularity in the methods is shown by total counts when it is not shown by coliform estimations.

In dealing with a milk supply normally having low bacterial counts, contamination from certain sources may be more evident from coliform counts than from total bacterial counts, and with raw milk supplies intended to be of excellent bacteriological quality, coliform counts may be an additional protection beyond that given by total counts. When they are used, it is advisable to determine whether representative cultures are *Escherichia, Aerobacter,* or intermediate types. Stuart *et al.*[46] found that the coliform organisms from certified milk showed considerable variation in the proportion of the three types, with a marked predominance of *Aerobacter* and intermediate forms; these would be less objectionable than *Escherichia* types, although still undesirable.

Infected udders are such an infrequent source of significant numbers of coliform organisms in milk that they can be ignored unless detailed study shows no other cause for relatively high coliform counts.

TESTS FOR SPECIFIC PATHOGENS

The most important information with reference to milk, cream, ice cream, butter, cheese, or any other food product is whether it contains pathogenic organisms. The difficulties in detecting such organisms in dairy products are so great that tests for them rarely are used in a routine way. Methods of examination for certain pathogens, notably *M. tuberculosis* and *Brucella* species, yield accurate results, but the methods for others are so unsatisfactory that negative findings do not admit of conclusions. The methods that are satisfactory are limited in their usefulness by the long periods required before the results are available or by the small volumes of milk that can be examined. Moreover, the absence of a particular pathogen in milk from a certain source on one day is no assurance that it will continue to be absent. Because of the number of pathogenic species that are spread through milk, an examination for each involves an excessive amount of work that is not justified in view of the inadequacy of many of the methods and the limited conclusions that can be drawn.

TESTS FOR UDDER INFECTIONS

Udder infections are widespread among dairy cows. Use of milk from such udders definitely is objectionable. The organism most commonly responsible for udder infections is *Streptococcus agalactiae* and it is non-pathogenic for man but, occasionally, the causative organism is a species that can produce disease in man and there is a hazard in consumption of milk in a raw condition. From the aesthetic standpoint, use of milk from infected udders is undesirable. Control of udder infections in herds also is advisable because of the economic losses involved in decreased milk production, rejected milk, etc. Maintenance of herds free from udder infections is receiving much attention.

Many cases of udder infections can be detected by physical examinations of the udders by a person familiar with udder anatomy. Such an examination is made most advantageously immediately after milking. However, not all cases can be detected in this way and, moreover, tests which can be applied to milk are desirable. The problem is complicated by two factors: (1) the infection may be very slight so that the secreting tissue is only slightly abnormal, and (2) there may be only a small portion of a quarter involved and milk from this is mixed with a much larger volume of milk from normal tissue. If milk from an infected quarter is mixed with milk from normal quarters, the problem is still further complicated, and it is advisable to examine milk from individual quarters; there also is an advantage in using milk soon after it is drawn so that changes due to bacterial action are excluded.

Various tests are applied to milk in an attempt to detect irregularities which indicate that it came from an abnormal udder. In some cases results of a test admit of definite conclusions but in others they do not and results of a combination of tests are advisable. Moreover, udders which are normal at one time may be infected later so that tests at intervals are desirable in any attempt to control udder infections in a herd.

Appearance of milk. Milk from an infected udder sometimes is abnormal in appearance. It may be watery, flaky, etc., and the color may be abnormal. Drawing the first few streams of milk from a quarter into a cup lined with fine wire screen or black cloth aids in detecting certain abnormal conditions. Use of a strip cup frequently is a part of each milking operation, especially in the production of milk of fine quality.

Acidity. In certain cases of udder infection the acidity of the milk is decreased, presumably due to infiltration of blood serum which is alkaline. Rosell[47] considers that if the acidity of milk from one quarter is less than 0.10 per cent and that from other quarters is higher, mastitis probably is indicated.

A colorimetric *p*H test involving use of bromthymol blue is extensively employed. It consists of adding the indicator to a small amount of milk and observing the color. Hucker *et al.*[48] suggest 2 drops of a concentrated alcohol solution to 3 ml of milk, and other investigators employ various concentrations. Within reasonable limits, concentration apparently makes little difference, but the indicator solution should be adjusted to approximately the neutral point by addition of sodium hydroxide. A yellowish-green to greenish-yellow denotes a normal reaction, a bluish-green a suspicious reaction, and a blue color a decidedly positive reaction. In some cases paper impregnated with bromthymol blue has been used for the test; milk is dropped on the paper or the paper dipped into the milk. Bromcresol purple also has been used as an indicator for determining the *p*H of milk.

A reaction distinctly more alkaline than normal milk indicates an udder infection, but a negative test is no assurance that the udder is normal. Shaw *et al.*[49] consider the *p*H test extremely unreliable in detecting cows with chronic mastitis. Fay *et al.*[50] reported the bromthymol blue test detected only 21.1 per cent of samples from quarters known to be infected. In certain cases milk from an infected udder is more acid than normal milk.

Catalase. The catalase content of milk from infected udders often is higher than normal, the high value evidently being associated with a high content of leucocytes. Various methods of determining the catalase content of milk are used; in general, they consist of adding hydrogen peroxide to milk, collecting the liberated oxygen in a tube, such as a fermentation tube, and measuring it.

Rosell[47] suggests a rapid catalase test in which 2 or 3 drops of hydrogen peroxide are mixed with 5 or 6 drops of milk on a flat piece of glass over a dark background. Appearance of bubbles in a few minutes is considered positive. In another rapid test hydrogen peroxide and milk are mixed in a test tube and the rapidity of gas formation observed. Various standards have been used in interpreting results of catalase tests. In general, a catalase content distinctly higher than normal indicates an udder infection, but milk from certain abnormal udders does not contain an excessive amount of the enzyme. Although some bacteria produce catalase, they are of no importance when fresh milk is studied.

Chlorides. In cases of mastitis the chloride content of milk often is high. Various procedures have been used for determining the chlorides in milk and various standards have been proposed; some of the titration methods give values that are higher than the actual chloride content, as determined on an absolute basis.

Sharp and Struble[51] noted that the chloride content of the milk of healthy cows fell very rapidly during the first few days of lactation, reached a minimum, increased slightly during the first 60 per cent of the lactation period, and then increased more rapidly, particularly during the last 10 per cent. A definite relation was found between the chloride value and the production of milk by healthy cows; an increase in the chloride content began when the production of an animal fell below certain limits. In the case of milk from infected quarters the first and last milk was high in chlorides, whereas the middle portion often approached or fell within the range of milk from normal udders.

The value of chloride determinations in detecting milk from infected udders is limited because of the other influencing factors, and some investigators consider it very unreliable.

Lactose. In milk from infected udders the lactose content, as determined with the usual chemical methods, often is low. A lactose content under 4 per cent sometimes is considered to indicate an abnormal udder. Rosell[47] reported milk which contained no lactose.

Body cells. Milk normally contains cells that come from the body of the producing animal. Some are contributed by the secreting tissue, some by the linings of the milk ducts, milk cistern, and teat canal, and some by the surface of the teat and udder, but the majority are cells that, because of their general appearance, commonly are considered to be leucocytes. The leucocytes are largely of the polymorphonuclear type but other types also occur; occasionally, cells containing bacteria are observed.

Excessive numbers of body cells often are associated with udder infections. However, the relationship between abnormal udder conditions and numbers of body cells in the milk is not a definite one, and cell determinations alone may admit of only limited conclusions, although they are valuable when used to supplement other evidence. Before the variations that occur in the numbers of cells in normal milk were recognized, milk from excellent dairies occasionally was excluded from certain cities because the cell counts were believed to indicate abnormal udders.

Methods for body cell counts. Various methods have been used to determine the numbers of body cells in milk.

The procedure for the microscopic count of the bacteria in milk is applicable to the counting of body cells and has largely superseded the other procedures. The general method was first employed for this purpose by Prescott and Breed [52] who pointed out that when milk is centrifuged many of the cells are carried into the cream, and that the percentage of cells thrown into the sediment is extremely variable. In the Doane-Buckley method,[53] the body cells in milk are concentrated by centrifuging and then counted in a definite volume of the concentrate by means of a blood cell counting apparatus. Since the procedure originally was devised the necessity of heating the milk to prevent fat globule clusters from holding many of the cells has been established by various investigators. In the Stewart-Slack method,[54] 2 ml of milk is centrifuged in a tube closed at each end with a rubber stopper, using a special centrifuge. The stopper is removed from the cream end, the cream layer broken with a wire, and the milk poured out. If the other stopper then is carefully removed, the sediment will adhere to it. The sediment is smeared over a definite area, usually 4 sq cm, with the aid of a small amount of water, and the slide quickly dried and stained with methylene blue. The average number of body cells or of bacteria per standard microscopic field is determined and the results expressed directly or calculations made to a milliliter basis. Many of the body cells and bacteria are carried into the cream layer so the results are more valuable on a comparative than an absolute basis. The Trommsdorff method involves use of a special centrifuge tube which is drawn out at the bottom to a fine bore and calibrated. Ten ml of milk is whirled in this for 10 min with a centrifuge and the percentage of sediment then read off directly. The method gives no information on the composition of the sediment so that when a high percentage is found a microscopic examination should be made. The procedure is relatively satisfactory for detecting excessive numbers of body cells in milk, such as are found occasionally with milk from individual quarters.

Number of cells in normal milk. The numbers of cells which various investigators have considered the maximum for normal milk differ greatly and over the years there has been a lowering of the numbers. At one time counts of 1 or more million per milliliter were regarded as normal by certain investigators. A standard of 500,000 per milliliter is now used in many cases when applied to milk from individual quarters. Rosell[47] considers that more than 250,000 per milliliter is to be taken as an indication of mastitis. Cherrington *et al.*[55] concluded that milk from normal udders usually has less than 50,000 cells per milliliter, whereas milk from infected udders almost invariably contains more than 100,000.

Milk from different quarters of a normal udder shows considerable variation in body cell content, and there also is a variation from day to day in the cell content of milk from a given quarter.

Variations in different portions of a milking. The different portions of a milking show variations in the cell counts that often are pronounced. In general, strippings run considerably higher than other portions of the milking, and fore milk may be either higher or lower than middle milk. In 15 comparisons Ross[56] found that on the average strippings had a cell content 53.85 per cent greater than fore milk and 52.14 per cent greater than middle milk. Russell and Hoffmann[57] studied 18 animals and noted that on the average strippings contained three times as many cells as fore or middle milk. In the results reported by Campbell,[58] strippings usually showed the highest cell count, although occasionally fore milk was high.

The higher count obtained with strippings than with other portions of a milking may be due to a tendency for the manipulation of the udder during the stripping process to dislodge cells so that they are more easily carried out with the milk.

Influence of stage of lactation. Colostrum commonly has a higher cell content than the normal milk secreted later. The following cell counts on colostrum and normal milk, respectively, taken from the studies of Ross,[56] illustrate this relationship: 713,500 and 18,000 per ml; 3,935,000 and 13,000; 500,000 and 58,000. Ross stated that, "Apparently most of the cells present in colostrum are broken-down epithelial cells," whereas Breed[59] reported that, "No cells of a different type than those formed in later stages of lactation were found in the colostral milk." Breed tabulated, according to the month of lactation, the results of 246 determinations of the cell content of milk from

126 different animals of various breeds. The average cell counts obtained on the milk of animals near the end of lactation did not seem to be markedly higher than those for the earlier months. However, several very high counts were obtained from animals advanced in lactation and the evidence indicates that high counts are more common then than during the height of lactation.

Influence of various factors. Results obtained by Russell and Hoffmann[57] showed a marked tendency for the cell content of milk to increase with an increase in the age of the cow. This may have been due to a greater opportunity for development of abnormal udder conditions with the animals that had been through a number of lactation periods.

In studies on the effect of various methods of drying up cows, Wayne and Macy[60] found that incomplete, intermittent, and incomplete intermittent milking, as well as complete cessation of milking, did not appear to have any marked effect on the cell content of the milk in subsequent lactation periods. Incomplete and intermittent milking usually resulted in somewhat higher cell counts during the periods of use. The cells observed in the milk during the drying up process were large, mononuclear cells.

It appears that machine-drawn milk sometimes has higher cell counts than comparable hand-drawn milk; this may be related to leaving the machines on the cows for excessive periods. Hucker[61] reported that milk from cows milked by machine gave an increased number of cells over comparable milk from cows milked by hand. Cone[62] found that, under the conditions used, milk from most of 31 cows had higher cell counts and also chloride values when the animals were machine milked than when they were hand milked; this was true with both infected and non-infected quarters. There was a marked difference between cows in their response to machine milking. During the investigation, little attention was given to the periods the machines were left operating, although the general tendency was to remove them as soon as the milk flow ceased.

Influence of condition of udder. An infection of the udder is the important factor causing a high cell content in milk. Ross[56] noted an instance in which a cow developed an abnormal quarter and milk from this had a cell count of 62,400,000 per milliliter, while that from the other three quarters contained 407,000. Russell and Hoffmann[57] reported results of cell counts on milk from three cows, each of which had an abnormal udder during part of the examination period. The highest cell count obtained with milk that showed evidence of garget was 3,625,000 per milliliter and the lowest was 489,000. Cherrington *et al.*[55] noted that the average cell count on milk from six normal cows was 43,000 per milliliter, whereas that on milk from seven diseased cows was 3,000,000; the maximum count on milk from individual quarters was 37,400,000 per milliliter. Many other examples of high cell counts on milk from diseased udders have been recorded by various investigators.

When milk has an excessive cell count it ordinarily has come from an infected udder. However, in some instances milk from an abnormal udder does not have a high cell count and the numbers may even be relatively low. The interpretation of intermediate cell counts presents difficulties, and various other tests often are used to supplement the cell counts in an attempt to establish the condition of the udder. Examinations for streptococci are of special importance in this connection.

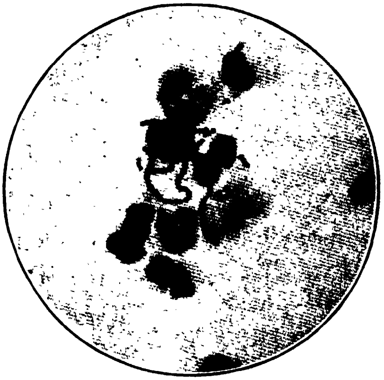

Fig. 3. Streptococci and leucocytes in milk from an abnormal udder.

Hoffmann[63] found that the injection of such inert materials as physiological salt solution, boric acid solution, and distilled water produced a rapid and marked leucocytosis, as shown by swelling and fever of the quarters injected and by increased cell content of the milk. A considerable number of the samples of milk showing high cell counts were reported as abnormal, but abnormal milk also gave counts that could not be considered excessive and it appeared that there is no close relationship between the cell content and an abnormal condition in the milk. The highest count reported by Hoffmann in the work on experimental leucocytosis was 25,400,000 per milliliter; this was obtained on abnormal milk coming from a quarter that had been injected the previous day with 300 ml of sterile distilled water. The next highest count observed was 18,250,000 per milliliter; it was obtained on abnormal milk coming from a quarter injected the previous day with 150 ml of sterile 2 per cent boric acid solution.

Influence of clarification and separation on cells. Clarification or separation removes a portion of the cells in milk (Chapter 5). This effect must be recognized when cell counts are made on milk that has been handled in a plant, since the number of cells originally present may have been materially reduced. Occasionally, a farmer reports that the amount of slime collecting in the separator bowl suddenly is increased; most often this is due to an excessive number of cells in the milk as a result of one or more udder infections.

Streptococci. Because of the frequency with which udder infections are due to streptococci, milk sometimes is examined microscopically for this morphologic type. Large numbers in freshly drawn milk, or in milk held so that there has been no growth of bacteria in it, are indicative of udder infections, especially when accompanied by high body cell counts; under these conditions the streptococci commonly are in long chains. Samples of milk from infected udders sometimes can be recognized immediately by the combination of long-chain streptococci and many body cells in microscopic preparations.

Because streptococci causing mastitis may be present in milk in small numbers, milk sometimes is incubated 12 to 24 hr at 37 C (98.6 F) and examined microscopically. With this procedure streptococci may multiply and be detected easily. If the milk is collected aseptically there is little danger of contaminants leading to erroneous conclusions but if the results are in question it is advisable to isolate and identify the streptococci which have multiplied. In some cases streptococci are present in normal udders; since these may include *S. agalactiae,* results must be interpreted with care. Some investigators have added certain chemicals to milk samples to be incubated with the idea of controlling growth of contaminants.

Plating of samples of milk, usually on blood agar, is another method of examining milk for streptococci. The procedure often is followed when a milk supply appears to be involved in the spread of septic sore throat, scarlet fever, or some other disease associated with streptococci. It also can be used in examining milk for *S. agalactiae.*

It should be recognized that mastitis may be due to organisms other than streptococci; these include staphylococci as well as organisms of types much less commonly related to infections so that failure to find streptococci is no assurance that the udder is normal.

Hotis test. The Hotis test was described by Hotis and Miller[64] as a rapid means of detecting *S. agalactiae.* More recently the importance of other organisms as a cause of mastitis has been recognized and some of them also can be shown by the test.[65]

In the test, 9.5 ml of milk, which should be collected with all precautions against contamination, is mixed with 0.5 ml of sterile 0.5 per cent bromcresol purple in a sterile test tube having an outside diameter no greater than 15 mm. The sample is incubated 20 to 24 hr at 37 C (98.6 F); longer incubation does not improve the results materially and may allow small numbers of contaminating bacteria to increase and interfere with the test. The fresh mixture of dye and milk normally has a purple color. If, due to mastitis, the milk is more alkaline the original color of the mixture shows this. Cone and Grant[66] noted the importance of the original color of the mixture in showing irregularities in milk.

After incubation, the color of the mixture may be unchanged or it may show various conditions as a result of the growth of organisms causing mastitis. With *S. agalactiae* there commonly is the formation of yellow flakes or clumps along the sides of the tubes and often a coarse flocculent sediment; the color of the milk also is changed as a result of acid formation. Other organisms produce changes of various types that may be rather characteristic. Rust-colored colonies are indicative of *Staphylococcus aureus.*

To avoid action of contaminating organisms in obscuring positive results with the test, certain chemicals have been added to the milk or the dye solution. Sodium azide has been used. Equal volumes of 0.2 per cent aqueous sodium azide solution and 0.5 per cent solution of bromcresol purple are mixed and 0.5 ml of the mixture added to 9.5 ml milk. Miller[65] noted that with azide growth of contaminants in Hotis tests was greatly repressed for at least 48 hr and streptococci continued to grow. However, the changes were not always typical and 48-hr incubation may be required for positive results. Also, staphylococci do not grow well in the presence of the azide. Accordingly, some of the information that might be obtained with the test is lost when the azide is used.

Miller[65] reported that the test was 85 to 90 per cent as effective as cultures on blood agar plates in detecting *S. agalactiae* in milk but only a small percentage of milk samples containing *Streptococcus dysgalactiae* and *Streptococcus uberis* were positive. *Staph. aureus* was readily identified.

Whiteside test. Whiteside[67] found that on addition of 2 ml of normal sodium hydroxide to 10 ml of milk from a cow having mastitis, with subsequent beating of the mixture with a glass rod, a viscid mass was formed. The reaction took place immediately at room temperature but failed if the mixture was brought to the boiling point. The test was positive before the bromthymol blue and strip cup tests and was most marked during the acute stage of the infection.

Murphy and Hanson [68] modified the test. They used 5 drops of fore milk and 1 drop of normal sodium hydroxide on a flat glass plate with a dull green blotter as a background. After mixing for 20 sec with a glass rod the material is examined by reflected light for the presence of a precipitate. Amount and character of the precipitate can be graded. The test paralleled closely the leucocyte count in ability to detect the presence of udder infections. The authors consider it to be a simple, accurate presumptive test for the presence of chronic mastitis.

Dunn *et al.*[69] concluded that leucocytes are directly or indirectly responsible for the Whiteside reaction in mastitic milk. They postulated that the protein material of the leucocytes reacts with sodium hydroxide to form a gelatinous mass.

Curd tension. Although curd tension determinations ordinarily are not used in attempts to detect mastitis, the effect of mastitis on the curd tension of milk is of interest because soft curd milk may be desired for infants and invalids (Doan [70]).

Mastitis, including subclinical mastitis, may cause a decrease in curd tension of milk from the infected quarter or quarters. Extent of the drop may vary with the degree of the infection. Effect of mastitis on curd tension of milk is shown by comparisons of milk from normal and infected quarters of the same cow. Hansen *et al.*[71] noted that mastitis caused by streptococci invariably lowered the curd tension of milk but that mastitis due to staphylococci apparently had no appreciable influence on it. Anderson *et al.*[72] reported that the three principal groups of organisms associated with chronic mastitis (two types of streptococci and staphylococci) were about equally effective in reducing curd tension, but that coliform infections decreased the curd tension more than any other type. According to Sanders *et al.*,[73] mastitis caused a decrease in rennet curd tension. There was a tendency for the properties of mastitis milk to return to normal when the bacteriological and pathological evidence of the infection subsided.

The general results indicate that in the selection of soft curd milk care should be taken to avoid cows with mastitis.

General considerations. An udder infection often can be detected readily by observations or tests on the milk. However, in some cases a decision as to whether the udder is infected is very difficult. Many special tests have been devised to aid in making a decision which indicates that previous procedures were not entirely satisfactory. With a variety of organisms responsible and with great variations in extent and degree of infection, the detection of udder abnormalities may be complicated. The presence in normal udders of organisms that frequently are associated with infections adds to the difficulties.

Mild infections present the most difficult problem and undoubtedly are easily missed. By using an enrichment method, Mattick *et al.*[74] demonstrated many cases of infection with small numbers of *S. agalactiae* in animals which gave negative results with other methods. Frequency of examination had an important influence on the numbers of cases discovered. Light infections were found to persist for long periods. Light infections also were found with *S. dysgalactiae* and with *S. uberis*. In 21 per cent of the animals *S. agalactiae* was present in their first lactation, and 4.5 per cent of heifers harbored the organism while in isolation before joining the herd.

Mastitis is present in many herds in the United States. Frayer[75] stated that it would be difficult to find a herd in Vermont or any other state in which one or more mastitic cows could not be located. In Michigan, Johnson and Bryan[76] checked 863 animals in 83 herds for the presence of streptococcic mastitis by examination of individual samples of milk; 21.9 per cent of the cows and 78.3 per cent of the herds were infected. The wide distribution of the disease makes it an important economic problem that also has public health implications.

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Chapter 3

Milk Fermentations

GENERAL CONSIDERATIONS

A milk fermentation is a process by which a change is produced in milk as a result of the activity of one or more species of micro-organisms. As milk normally leaves the udder, it is very satisfactory for the growth of many species and, accordingly, undergoes a series of changes; the processes producing the different changes constitute the milk fermentations. Many of the fermentations that occur in milk also occur in various milk derivatives.

Normal and abnormal fermentations. The common change occurring in milk is development of lactic acid with the resulting coagulation of the casein. The process bringing this about is designated lactic acid fermentation or the souring of milk. Because of the regularity with which it occurs under the usual conditions, it sometimes is called the normal fermentation of milk. The processes causing other changes, such as sweet curdling, development of ropiness, gas, color, flavor, odor, etc., constitute the abnormal milk fermentations.

Ordinarily, a milk fermentation can be caused by more than one species and sometimes by a series of species. However, the most convenient basis for study of the changes occurring in milk as a result of action of organisms is a division into the different types of changes, rather than an attempt to classify all the causative species. The principal reason for this is that the change taking place is the important thing under practical conditions and, frequently, information with reference to a certain fermentation can be given without knowing the specific organism involved so that valuable time is saved in correcting the difficulty. If a consideration of the changes occurring in milk were based on a classification of the organisms responsible, the first step in dealing with any abnormal condition would be the isolation and identification of the causative species and this is very time-consuming. An objection to the plan of studying the different fermentations as units is that organisms not at all related often are thrown together because they happen to have one common character, such as development of gas or ropiness in milk; for example, a discussion of the gassy fermentation involves a consideration of such widely differing organisms as *Torula cremoris* and those of the *Escherichia-Aerobacter* group.

Mixed fermentations. It often happens that two or more fermentations occur in milk at the same time; production of acid may be accompanied by gas formation or a color change, development of ropiness may occur along with a change in flavor, or there may be various other groupings. Often these combinations of fermentations are due to the activity of more than one species but some organisms, in pure culture, produce two or even more changes in milk. The *Escherichia-Aerobacter* group produces gas, off flavors, and acid and certain cultures also produce ropiness so that it should be considered under at least four fermentations; while this group is an extreme example there are many species that produce more than one type of change.

Variations in organisms. Organisms that apparently belong to the same species may differ in a character that is important from the standpoint of milk fermentations. The best illustrations of this are in connection with ropiness; some cultures of *Lactobacillus bulgaricus* produce a ropy condition, whereas others do not, and this same variation occurs with a number of other species, including *Lactobacillus casei* and *Streptococcus lactis.* Variations in flavor production also occur among organisms apparently belonging to the same species.

Associative action. Associative action is the combined action of two or more organisms in producing a change which cannot be produced by one of the organisms alone. Action of the organisms growing together often can be traced to the influence of the products of one on rate of growth or products of the other, but in some instances no such simple explanation is justified.

The effect of organisms growing in combination is particularly important in dairy products and can influence amount of acid produced, development of ropiness, color production, volatile acid production, flavor development, etc. It is probable that associative action often is involved in the changes occurring in milk and cream on extended holding, especially changes in flavor. Failure to isolate an organism capable of producing a certain fermentation in a dairy product sometimes is due to the change being the result of action of organisms in combination. When a pronounced change in milk is due to associative action, the species involved often are present in considerable numbers so that isolations are not difficult.

LACTIC ACID FERMENTATION

The process by which lactic acid is produced in milk, cream, etc., through action of organisms constitutes the lactic acid fermentation. In milk and cream at favorable temperatures enough acid commonly is produced to precipitate the casein and the liquid thus becomes a semisolid mass. The lactic acid is formed from the lactose, and various species of bacteria are capable of bringing about the transformation.

Frequency of the fermentation. Lactic acid fermentation occurs regularly in raw milk in all parts of the world, its rate being determined primarily by the temperature at which the milk is held. Occasionally, in new dairy districts, souring of milk is markedly delayed; this probably is because the surroundings are less thoroughly seeded with lactic acid organisms than in the older districts so that the milk is but lightly contaminated. Lactic acid is produced through bacterial activity in various materials, such as silage and sauerkraut, in addition to dairy products.

Historical. Although living organisms were observed in sour milk early in the eighteenth century, it was not until 1837 [1] that they were suggested as the cause of the souring. The idea of the causal relationship of organisms to this change found few advocates until Pasteur,[2] in 1857, definitely showed the existence of lactic-acid-producing organisms. In 1878, Lister [3] succeeded in isolating one of these forms and thus provided incontrovertible proof. Before the importance of organisms in bringing about various decompositions was recognized, the change from lactose to lactic acid was thought by certain investigators to result from something in the casein.

Lactic acid. Lactic acid was discovered in sour milk by Scheele, in 1780, but it was not until many years later that its constitution was established as $CH_3—CHOH—COOH$ (α-hydroxypropionic acid). It is non-volatile and is miscible with water, alcohol, or ether in all proportions. Commercial lactic acid is a colorless, somewhat viscous liquid having a strongly acid taste and containing water. By distilling the aqueous solution under very low pressure (1 mm), lactic acid can be purified and obtained as a crystalline solid melting at 18 C (64.4 F); when heated at ordinary pressures in an attempt to remove the water, it is partially converted to the anhydride before dehydration is complete. Pure lactic acid is odorless.

Transformation of lactose to lactic acid. The transformation of lactose to lactic acid can be generalized as follows:

$$C_{12}H_{22}O_{11} \text{ (lactose)} + H_2O = 4C_3H_6O_3 \text{ (lactic acid)}$$

The first step is the hydrolysis of lactose to glucose and galactose. The changes taking place in the formation of lactic acid from these sugars usually are considered to be the following:

$$CH_2OH—(CHOH)_4—CHO \rightarrow$$

$$2CH_2OH—CHOH—CHO \text{ (glyceric aldehyde)}—2H_2O \rightarrow$$

$$2CH_3—CO—CHO \text{ (pyruvic aldehyde or methylglyoxal)} + 2H_2O \rightarrow$$

$$2CH_3—CHOH—COOH \text{ (lactic acid)}$$

Phosphorylation of the sugar molecule probably plays a role in the dissimilation.

The transformation of lactose to lactic acid is not quantitative and the fermentation yields other products, the so-called secondary products, which may include various acids, aldehydes, and alcohols. The nature and quantity of the secondary products vary greatly with the different organisms; the products are very important since they greatly influence the flavor of the fermented milk.

Optical isomers of lactic acid. Lactic acid is known in the three isomeric modifications that are indicated by the asymmetry of the alpha carbon atom. Some organisms produce *d* acid only, others produce *l* only, and still others produce both *d* and *l*; of the organisms that produce both, some form the two in equal amounts, thus giving *i* acid only, whereas others form the two in unequal amounts and, accordingly, give *i* acid together with *d* or *l*. The isomeric modification produced appears to be constant for a given organism regardless of the holding temperature, although with organisms producing both *d* and *l* acid the one produced in the smaller amount may be absent under conditions unfavorable for growth. In naturally contaminated milk the form of the acid produced is influenced by the holding temperature because of the effect on the relative growth rates of the various organisms producing lactic acid.

Acidity of fresh milk. Freshly drawn milk shows considerable variation in the acidity, calculated as lactic acid, and in the *p*H. In 86 tests on individual samples from 31 cows, Sommer and Hart[4] noted acidities from 0.102 to 0.257 per cent; 45 of them were above 0.18 per cent. The *p*H values of more than 300 samples of fresh milk, each representing only a portion of the milking, were found by Van Slyke and Baker[5] to range from 6.50 to 7.20, with 80 per cent of the samples under 6.76. In general, with a decrease in acidity there was a marked tendency toward a decrease in specific gravity and in the percentages of fat, total solids, solids-not-fat, casein, and lactose, but an increase in the percentages of proteins other than casein and in ash and chlorine. With 20 cows the *p*H of the milk from different quarters of the udder commonly varied, although in most cases the variations were not large.

McInerney [6] reported acidities from 0.10 to 0.22 per cent with fresh milk from individual cows in two herds. Milk with high percentages of solids-not-fat and ash showed a high acidity, and milk with low percentages of these materials showed a lower acidity.

With the milk of a number of cows, Rice and Markley [7] noted variations in the acidities from 0.086 to 0.229 per cent and in the *p*H values from 6.898 to 6.432. In general, high-acid milk contained more nutrients and was particularly high in phosphates. The investigators concluded that carbon dioxide is responsible for 0.01 to 0.02 per cent acidity in milk, casein for 0.05 to 0.08 per cent, albumin for less than 0.01 per cent, and phosphates for the remainder, although citrates might account for 0.01 per cent. Robinson and Samson [8] concluded that inorganic phosphates are the primary cause of the acidity of fresh milk.

Houston [9] studied more than 2,500 samples of milk from 39 complete and 38 incomplete lactation periods. The average acidity for all the tests was 0.189 per cent and for the tests involving complete lactation it was 0.190. Average acidities for individual lactations ranged from 0.154 to 0.235, whereas acidities of individual milkings ranged from 0.105 to 0.270. The greatest difference in acidities of individual milkings of a cow during a lactation was 0.145; the acidities ranged from 0.250 at the beginning of lactation to 0.105 when the cow was almost dry. In general, the acidities were highest at the beginning of the lactation period and lowest at the end. The fall was not uniform but was fairly rapid during the first 8 weeks of lactation and also during the last 7 weeks.

Sommer [10] found the *p*H values on 386 samples of milk ranged from 6.22 to 6.77; both milk and udders were carefully examined to detect subclinical mastitis and the definite and doubtful samples eliminated.

Caulfield and Riddell [11] found the acidities of 811 samples from individual cows of various breeds ranged from 0.098 to 0.295 per cent. Average percentages for the various breeds were Ayrshire 0.160, Holstein 0.161, Guernsey 0.172, and Jersey 0.179, and the average for all breeds was 0.166. The acidity of colostrum was high, averaging 0.44 per cent for the first milkings from a group of 10 cows. At the third milking the acidity averaged 0.26 per cent, at the sixth milking 0.21

per cent, and there was a gradual decline during the first 15 to 20 days of lactation. With a group of 36 cows the acidities showed a gradual decline throughout the lactation period; the most pronounced changes were at the beginning and end of lactation, and from the second to the seventh month of lactation the acidities remained fairly constant. There was a marked decline in the average acidities during the last month of lactation but the extent varied widely between individual animals.

There often is some fluctuation in the acidity throughout a milking, the fore milk being comparatively low, the middle milk highest, and the strippings between the two. This is the variation that would be expected from the composition of the different portions of a milking, if account is taken of the fact that the fat carries little, if any, acid. Influence of the feed on the acidity of milk is apparently negligible; Turner and Beach[12] noted no effect from silage, and Sommer and Hart[13] added up to 120 ml of concentrated sulfuric acid to the ration of a normal cow without affecting the acidity of the milk. Duncombe[14] found that feeding lactic, butyric, acetic, or phosphoric acid to cows had no appreciable effect on the titrable acidity or *p*H of the milk, whereas when the cows were changed from a dry to a pasture ration there was a decrease in the titrable acidity but practically no change in the *p*H.

Changes in acidity of milk. When ordinary raw milk is allowed to stand at room temperature the acidity soon begins to increase as a result of activity of organisms. The acidity at which milk tastes sour varies with different persons and also with the initial acidity, a definite increase in acid being more important than the actual acidity. With most people, milk having an acidity of 0.25 per cent tastes sour and, with many, milk having definitely lower acidities tastes acid. Persons accustomed to judging flavors of milk and milk derivatives frequently detect an acid flavor at acidities below 0.20 per cent, especially when the milk had a low initial acidity. Schultz *et al.*[15] reported an acidity was first detectable by taste at a *p*H of about 6.0.

Certain flavors associated with development of acid may be detected with only very slight acidity increases. In these cases it is not the acid but the other compounds produced in the fermentation that are detected by the senses of taste or smell. Gould and Jensen[16] noted that acid-associated flavors were detected at very low levels of lactic acid formation.

As the acid in milk continues to increase it eventually causes a precipitation of the casein, the point at which this occurs varying with different samples of milk and usually falling between 0.50 and 0.65 per cent. Van Slyke and Baker[17] found that the casein begins to coagulate when the *p*H reaches 4.64 to 4.78. On boiling, milk commonly curdles at an acidity varying from 0.30 to 0.45 per cent.

With many lactic-acid-producing species the acidity continues to rise after coagulation of the casein until the accumulation of acid prevents further growth. The maximum amount of acid developed in milk varies widely with different species, and even with different cultures of the same species. The lactose, which averages about 5.1 per cent in milk, is very rarely, if ever, the limiting factor in acid production, and under ordinary conditions there still is considerable lactose remaining when development of acid has stopped. Van Slyke and Bosworth[18] found that in fresh milk inoculated with two common organisms, *S. lactis* and *Aerobacter aerogenes,* and allowed to stand at room temperature for 60 hr, 22 per cent of the lactose disappeared and 88.5 per cent of the amount used was converted to lactic acid.

The spontaneous souring of milk ordinarily involves chemical changes other than formation of lactic acid. Van Slyke and Hart[19] pointed out that the acid produced combines with the bases of some of the inorganic salts and then with the calcium that is in combination with the casein, which results in formation of a precipitate of free casein. As more acid is produced the free casein unites with it, forming a casein salt of the acid, and this constitutes the curd noted in sour milk. Bosworth and Prucha[20] found that the citric acid present disappears during the souring of milk.

According to Van Slyke and Baker[17] lactic acid exists in sour milk largely as lactate but partly as free acid; a portion of the latter is in solution and a smaller part, about 20 per cent in coagulated sour milk, is adsorbed by the casein. In freshly separated skim milk pasteurized at 62 C (143.6 F), cooled to 25 C (77 F), inoculated with *S. lactis,* and then held at 25 C, free lactic acid appeared in appreciable amounts after about 20 hr, when it equaled 0.1 ml *N*/10 acid in 100 ml of milk; it increased more rapidly in the next few hours and after 48 hr equaled 20 ml *N*/10 acid.

Preservative action of lactic acid. The lactic acid produced in milk prevents growth of many species of bacteria, especially putrefactive types, because of the lack of acid tolerance among these organisms. Thus lactic acid acts as a preservative. Rapid decomposition of sour milk does not occur until the acidity has been decreased. Under the usual conditions this decrease is brought about by various microorganisms, particularly molds, which use part of the acid and neutralize part with certain products of their metabolism. In raw milk allowed to stand at ordinary temperatures, development of acid, decrease in this acid largely through growth of molds, and then rapid decomposition constitute a fermentation cycle that occurs with great regularity.

However, with sour milk and cream, objectionable flavors other than those of a putrefactive nature develop on standing so that the holding period is more or less limited under ordinary conditions. This change is largely due to activity of micro-organisms, but chemical action probably also plays a part in certain instances. The micro-organisms capable of growing in the presence of considerable acid are of various types, with molds and yeasts of special importance; certain organisms even grow more rapidly in sour milk than in sweet milk.

Lactic acid organisms. At the time (1878) that Lister[3] made the first isolation of a lactic acid organism there still were many people who doubted the causal relationship of micro-organisms to fermentations. With development of a technic satisfactory for the study of bacteria, the action of different species in changing a portion of the lactose in milk to lactic acid was definitely established. These organisms vary widely in the quantities of acid produced and in their general characters. Some of them form relatively large amounts and production of lactic acid is the conspicuous change when growth occurs in dairy products; with others, production of lactic acid is not such an outstanding character and the organisms are of primary importance because of some other effect. If an organism is to be of significance in the production of lactic acid in milk, it must be capable of fermenting lactose. Species closely related to the typical lactic acid organisms are of little importance in milk because of their failure to ferment lactose, although they may ferment other sugars readily.

The bacteria, which are characteristically lactic-acid-producing organisms in that they attack lactose actively and produce lactic acid in relatively large amounts from the lactose fermented, are (1) *Streptococcus lactis* group and closely related forms, (2) *Streptococcus liquefaciens* group, (3) certain species of the genus *Lactobacillus,* and (4) certain species of the genus *Bacillus.* The organisms with which production of lactic acid is less important include various species. *Escherichia-Aerobacter* organisms form some lactic acid from lactose but they also produce gases and other compounds that ordinarily are of more importance than the acid. *Streptococcus paracitrovorus* produces lactic acid from lactose but the amount usually is small and the organism is of interest primarily because of its ability to ferment citric acid with formation of various compounds of value from the standpoint of the desirable flavor of certain dairy products. Since lactose is so important in milk and its derivatives, ability of an organism to ferment it immediately gives the species an added significance, regardless of whether large quantities of lactic acid are produced.

STREPTOCOCCUS LACTIS GROUP AND RELATED ORGANISMS

Certain of the streptococci play a striking role in the development of acid in dairy products. The most important of these constitute the *S. lactis* group but there are closely related forms that are of significance under some conditions. At favorable temperatures these organisms produce lactic acid in milk rapidly or fairly rapidly, without digestion or formation of gas; litmus, when present, commonly is reduced rapidly and more or less completely.

1. *Streptococcus lactis* GROUP

The organism isolated by Lister (1878)[3] and named *Bacterium lactis* by him belongs to what is now known as the *S. lactis* group. Leichmann (1896)[21] proposed the name *Bacterium lactis acidi* for what undoubtedly was the same organism, and Kruse (1903)[22] suggested *Streptococcus lacticus* because he considered the organism a *Streptococcus*. Various other designations also have been proposed. *S. lactis* is the name now commonly used, since it retains the species name given by Lister and replaces the original generic name with one commonly employed in systems of nomenclature.

Variations in group. *S. lactis* refers to a group of organisms, the members of which show variations in the flavor produced, consistency of the fermented milk, rate of reduction of litmus, rate of acid production, etc. These differences are of significance because of the frequency with which the organisms develop in dairy products. On the basis of variations commonly encountered, Hammer and Baker[23] suggested a number of varieties of *S. lactis* and, undoubtedly, there are others of importance. The varieties proposed are:

S. lactis var. *maltigenes* is characterized by the production of a malty or caramel flavor in milk, cream, etc. In some instances this condition has been erroneously ascribed to excessive pasteurization exposures, but it differs definitely from the flavor due to high heat. There are variations among strains in the intensity of the flavor. The organism can produce a malty condition with no significant increase in acidity, and sweet milk or cream sometimes is distinctly malty, although sour products are more commonly involved. Frequently, the organism is present in such small numbers that it does not influence the flavor. Production of a malty flavor is a very stable character that persists through many transfers of a culture; it occurs in media other than milk and cream when the organism grows well in them. Sadler [24] reported that the compound responsible for the malty flavor is ether soluble. *S. lactis* var. *maltigenes* is readily isolated from various materials showing a malty flavor unless they have been heated and, occasionally, is encountered in normal products. A malty flavor in a dairy product is not always due to this organism since Tracy and Ramsey [25] found it could be caused by a *Micrococcus;* when an organism of the *Bacillus subtilis* group was also present the flavor was more pronounced, whereas acid-forming bacteria retarded its development. Davis *et al.*[26] studied the organism producing a malty flavor in a milk supply and found it closely resembled *S. lactis.* It entered the milk from outside sources and in some instances also was present in the cows' udders.

S. lactis var. *hollandicus* produces a ropy or slimy condition in milk, cream, etc. There are great variations among strains in extent of the ropiness, some producing only slight ropiness, others producing a conspicuous condition. The ropy character is not stable; a culture often varies from time to time in the extent of the ropiness produced and may lose entirely the ability to cause ropiness, especially when grown at a relatively high temperature. Hammer [27] obtained non-ropy cultures in small numbers from ropy ones by plating and picking colonies, and also obtained a few ropy cultures from non-ropy ones in the same manner. The microscopic pictures accompanying ropiness vary greatly. Some ropy cultures show chains of cells, others show clumps, and still others show great numbers of cells in pairs. *S. lactis* var. *hollandicus* often can be isolated from ropy lactic acid culture or sour cream but is unusual in non-ropy materials.

S. lactis var. *anoxyphilus* differs from the typical *S. lactis* in that it reduces litmus and comparable indicators slowly. Instead of rather completely reducing litmus milk before coagulation begins, the milk is partially or completely coagulated before reduction. Finally, reduction is practically complete and the culture then has the usual appearance of an *S. lactis* culture so that unless observations are made at the proper period the slow reduction is not detected. The character is a stable one and persists in a culture over extended periods. It does not appear to be of any practical significance. *S. lactis* var. *anoxyphilus* occasionally is isolated when various dairy products are plated and colonies picked.

S. lactis var. *tardus* coagulates milk more slowly than the other varieties of *S. lactis*. There is considerable variation in the coagulation rates of different strains. In general, the delayed coagulation is due to relatively slow multiplication of the cells and the extent of the delay is greater at 21.1 C (70 F) than at 37 C (98.6 F). By plating and picking colonies, cultures of *S. lactis* var. *tardus* often can be obtained in small numbers from cultures that coagulate rapidly. Harriman and Hammer[28] found this variation occurred regardless of how many times the rapid cultures were purified by plating. The slow coagulation rate of *S. lactis* var. *tardus* is very stable and this variety does not yield rapidly coagulating cultures when it is plated. The organism often is isolated when sour milk or cream is plated and colonies picked.

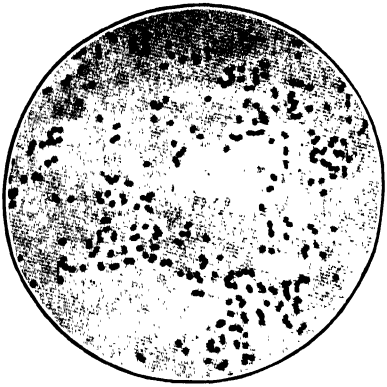

FIG. 4. *Streptococcus lactis* in milk.

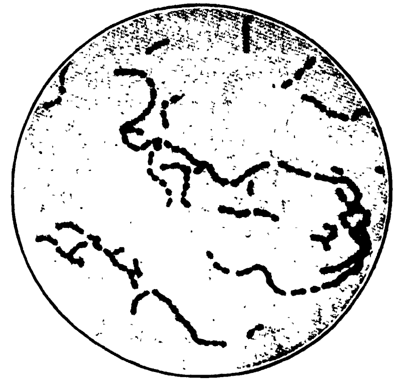

FIG. 5. *Streptococcus lactis* showing long chains. Iowa Agr. Exp. Sta. Res. Bul. 22.

Morphology. The names that have been proposed for the organism now designated *S. lactis* indicate that there has been a difference of opinion as to the morphology. At present it is generally considered a practically spherical form that may be slightly longer than broad, especially just before division. Size of the cells in different cultures varies and may be influenced by age of the culture, food materials present, temperature of growth, etc.; the diameter usually falls between 0.4 and 1.0 micron. When grown in milk the cells ordinarily are arranged in pairs or short chains, although cultures showing long chains occasionally are encountered; rarely, pairs of cells are grouped into clumps. In liquid media other than milk, chains may be formed, but on solid media they are unusual. The organism is Gram positive and stains readily with ordinary stains, is non-motile, and does not form spores. Capsules often are present although usually found on only a portion of the cells in a preparation.

Cultural characteristics. *S. lactis* grows well in milk and certain milk derivatives. It develops fairly well on standard, tomato, or whey agar. Growth in bouillon is meager, unless some material that the organism is capable of fermenting is added. The most characteristic of the cultural reactions is that in litmus milk. Ordinarily, the first observable change is a reduction of the litmus which usually begins at the bottom and progresses toward the top; reduction of the litmus often is practically complete before any coagulation is evident, although this is not the case with *S. lactis* var. *anoxyphilus.* Coagulation also commonly begins at the bottom, and when this is complete it is usual to find the milk white, except for a narrow pink band at the surface. As the culture ages this pink band gradually increases in width until the whole tube is pink. There is no digestion or gas formation.

Colonies on agar are small, white, smooth-edged, and non-viscous. Commonly, surface colonies are round and subsurface colonies ellipsoid.

Biochemical characteristics. The organism, *S. lactis,* ordinarily produces acid from glucose, galactose, fructose, lactose, and maltose; other common test materials may or may not be fermented. Lactic acid regularly is produced from the lactose in milk, and at favorable temperatures the rate is rapid; volatile acids, mainly acetic and propionic, also are formed in small amounts. The maximum acidity developed in milk by most cultures is between 0.7 and 1.0 per cent, although occasionally an acidity up to 1.25 per cent is encountered. The percentage of the theoretical yield of lactic acid (according to the reaction $C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3$) that is produced by *S. lactis* varies from 88 to 98 per cent. The lactic acid formed is of the *d* type, regardless of the temperature at which the fermentation occurs.

White and Sherman [29] found that under strictly aerobic conditions, obtained by vigorous and complete aeration, the percentage of lactic acid produced by *S. lactis* per unit of glucose fermented ranged from 11 to 63 per cent; anaerobic controls regularly gave more than 95 per cent lactic acid from the glucose fermented.

By chemical means an increase in the amount of soluble nitrogen in milk cultures often can be detected, and the increase is greater if calcium carbonate is added to the milk and frequently distributed by shaking during the fermentation to neutralize some of the acid formed; the protein breakdown goes to the amino acid stage. Cultures of *S. lactis* var. *tardus* may not increase the soluble nitrogen in milk.

Growth conditions. *S. lactis* grows over a wide temperature range, with different cultures showing somewhat different temperature extremes. Many cultures are most active between 30 and 40 C (86 and 104 F) although development also is relatively rapid at 21.1 C (70 F). Growth is comparatively slow at 10 C (50 F), commonly does not occur at 45 C (113 F), and a few cultures also fail to grow at 37 C (98.6 F). In general, the maximum acidity produced is somewhat higher at 21.1 C than at 37 C, although the period required is longer. The organism is facultative; ordinarily, acid production is somewhat more rapid with a restricted than with an abundant air supply. *S. lactis* is easily destroyed by heat. Milk cultures die rather quickly because of the destructive action of the acid formed.

Fermenting capacity of *S. lactis* cells. The lactic acid produced per individual cell per hour in milk by *S. lactis* was found by Baker *et al.*[30], using the direct microscopic count, to lie between 5×10^{-10} and 10×10^{-10} mg for a vigorous culture; the rate tended to decrease as the curdling point was approached. Considerable variation occurred in the rate of acid production with different cultures. The ratio of the total acid developed to the number of organisms present was fairly constant. There was a tendency for the acid contained, whether measured as titrable acidity or as *p*H, to increase geometrically until coagulation.

At one time growth and acid production of *S. lactis* were considered to be more or less independent. Recent evidence indicates, however, that there is acid developed during the early growth period, but that it is difficult to detect because it is so small. No relationship exists between the acidity and the number of cells of *S. lactis* in milk in which this organism is growing because, after the acidity has reached a certain point, the cells begin to die, owing to their inability to tolerate the acid, while the acid production may still continue slowly.

Enzyme action in acid production. Action of an enzyme in the production of acid by *S. lactis* was demonstrated by Hastings *et al.*[31] Milk was heated to destroy the inherent milk enzymes, inoculated with *S. lactis,* and after the organism had grown for a time preserved with 3 per cent toluene. There was a definite increase in acid over an extended holding period, although the final acidity was comparatively low. Inoculation experiments showed that growth could not have persisted after addition of the toluene and that the organisms were destroyed within a short period; microscopic examinations indicated an eventual disintegration of the cells.

Acids, other than lactic, formed by *S. lactis.* The distinct acid odor of most cultures of *S. lactis* suggests that acid other than lactic has been formed and determinations on cultures show a definite but rather small amount of volatile acid. Evans [32] reported that cultures of *S. lactis* produced a fairly constant quantity of acetic acid in milk, equivalent to about 0.12 gm per liter. Hammer and Sherwood [33] found that the volatile acid produced by *S. lactis* was made up of acetic with considerable amounts of propionic.

Associative action involving *S. lactis.* Various organisms common in dairy products influence the amount of acid produced in milk by *S. lactis.* Marshall and Farrand [34] found that 24 of 42 organisms isolated from milk accelerated the growth, 11 had no effect, and 7 retarded it. The acceleration occurred with various organisms and at different stages in the development of *S. lactis;* apparently, it was due to products formed by the associated organism, and these sometimes were stable to heat and sometimes not. In mixtures of *S. lactis* and other organisms, the latter sometimes disappeared and sometimes persisted and caused abnormal lactic acid fermentations. In certain instances the relative numbers of *S. lactis* and the other organism had a decided influence on the acceleration of growth of *S. lactis.* When certain yeasts are grown in combination with *S. lactis* in milk, the period it retains its vitality is greatly increased.[35] Cox and Whitehead [36] reported that *Bacillus subtilis* appeared to stimulate production of acid by *S. lactis,* whereas *Escherichia coli* caused a decrease in some experiments and an increase in others; two additional species had only slight effects.

S. lactis inhibits growth of various bacteria in milk, presumably through production of acid. It also may inhibit an organism which produces considerable acid, since Rogers [37] noted that in mixtures of *S. lactis* and *L. bulgaricus* containing a predominance of *S. lactis,* the *Lactobacillus* type was definitely suppressed by a specific substance diffusible through a collodion membrane; the substance was not destroyed by boiling, but was at least partially removed by filtering through an earthenware or plaster of paris filter. The same substance apparently also limits growth of *S. lactis* itself.[38]

Importance of *S. lactis.* *S. lactis* is the species mainly responsible for the souring of milk and cream under practical conditions. In this way it causes great economic losses since sour milk and cream cannot be used for many purposes for which the sweet products can be employed and, accordingly, are less valuable. *S. lactis* var. *maltigenes* also produces an objectionable flavor in milk and cream.

S. lactis plays a necessary part in the preparation of certain dairy products and, therefore, is beneficial as well as harmful. It is important in preparation of lactic acid culture, in ripening of cream for buttermaking, in manufacture and ripening of many cheeses, and in making of various fermented milk products, its principal function in all these cases being the formation of acid.

Distribution and sources of *S. lactis*. At the time milk leaves the stable it practically always contains *S. lactis* but the percentage of the total flora made up of the organism is small. Ordinarily, it does not come from the interior of the udder, and in experiments involving inoculation of *S. lactis* into the udder through the teat canal the organism quickly dies out so the interior of the udder apparently is unsuited to its development. However, Leitch[39] reported that in several outbreaks of a burnt flavor in milk, which apparently were due to *S. lactis* var. *maltigenes,* the causative organism was present in the milk of certain animals; in one herd of 37 cows, 7 had pronounced intramammary infections, and 4 were lightly infected.

Probably the most common source of *S. lactis* under practical farm conditions is the utensils and often it can be obtained from the coat of the cow, silage, and various grains. Esten[40] found it in the feces of 11 of 25 cows and obtained it from the mouths of 17 of 21 cows; mangers and materials within reach of a cow's mouth were well seeded with it. McGuire[41] found the organism a normal constituent of cow feces. Stark and Sherman[42] repeatedly isolated *S. lactis* from certain plants, but not from all plants examined, and did not find it in mouths or throats of cows, in bovine or human feces, or in soil. They suggested that plants may represent the natural habitat of the organism.

Isolation of *S. lactis*. *S. lactis* is easily isolated by allowing raw milk or cream to sour at ordinary temperatures, plating on a suitable agar, incubating the plates at 21.1 C (70 F), and then picking colonies into litmus milk. In recently soured milk *S. lactis* ordinarily makes up 90 to 99 per cent of the total flora so that most of the colonies picked will be *S. lactis,* even when no attempt at selection is made. Colonies of *S. lactis* are easily distinguished from those of many, but not all, of the organisms common in milk. When an attempt is made to isolate *S. lactis* from fresh sweet milk by direct plating, difficulty frequently is encountered because of the small numbers in which the organism is present and the lack of anything characteristic about the colonies.

Action of *S. lactis* on animals. In experiments at the Iowa Agricultural Experiment Station, various milk cultures of *S. lactis* inoculated into either rabbits or guinea pigs were not harmful. Cultures injected into rabbits intravenously could be recovered from the spleen and liver for some hours after inoculation, but repeated passage through rabbits in this way failed to yield cultures capable of causing death in rabbits.

2. ORGANISMS CLOSELY RELATED TO *Streptococcus lactis*

Streptococcus cremoris. Certain investigators consider *S. cremoris* a species distinct from *S. lactis* on the basis of a tendency to form long chains in milk and the failure to ferment maltose and dextrin. It also may have a somewhat lower maximum growth temperature.

However, various cultures of lactic acid streptococci that show only pairs or short chains when first inoculated into milk tend to give long chains after being carried rapidly through many transfers. Sherman and Hussong[43] found that a culture of *S. cremoris* which was maltose negative yielded both maltose positive and negative strains when it was plated and colonies picked. More recently, Yawger and Sherman[44] reported that *S. cremoris* can be distinguished from *S. lactis* by its inability to produce ammonia in 4 per cent peptone or to grow at 40 C (104 F) in the presence of 4 per cent sodium chloride and in broth at a *p*H of 9.2. Hunter[45] has noted that tests with strains of bacteriophage tend to confirm a distinction between *S. cremoris* and *S. lactis.*

Certain investigators consider *S. cremoris* as the typical acid-producing organism of lactic acid cultures. However, in development of such cultures *S. lactis* is entirely satisfactory.

Streptococcus thermophilus. *S. thermophilus* differs from *S. lactis* primarily in its temperature relationships. It grows well at 45 C (113 F) and at 37 C (98.6 F), but growth is slow at 21.1 C (70 F) and does not occur at 10 C (50 F). The organism also is capable of surviving comparatively high heat exposures. Because of its temperature relationships, *S. thermophilus* is the important lactic acid *Streptococcus* encountered in milk and other dairy products that have been subjected to relatively high heat. It often is easily isolated from pasteurized milk, particularly if the milk is incubated at temperatures favorable for its growth, and is important in swiss cheese, which commonly is cooked at a high temperature.

S. thermophilus does not reduce the litmus in litmus milk as completely as a typical culture of *S. lactis,* and the top one-third or more of a tube may show a pink color, even when reduction is at the maximum. It produces *d* lactic acid.

Additional organisms. Various organisms essentially like *S. lactis* have been recognized as distinct species on the basis of fermentation tests and other minor variations. This practice results in a multiplicity of species that is very confusing.

Yawger and Sherman [46] reported the isolation from milk of four cultures of *S. lactis* which did not ferment lactose. These cultures agreed with typical *S. lactis* in all other respects.

STREPTOCOCCUS LIQUEFACIENS GROUP

Streptococcus liquefaciens rapidly coagulates milk and then actively proteolyzes it. The coagulation occurs at a low acidity and is due to a rennin-like enzyme. With 15 cultures studied by Long and Hammer [47] the titrable acidities at coagulation ranged from 0.25 to 0.29 per cent and averaged 0.27, whereas the *p*H values varied from 6.02 to 5.85 and averaged 5.90. Following coagulation the acidity continues to increase until it is essentially the same as that produced by *S. lactis*. Proteolysis is evident soon after coagulation. It increases and the mass of curd becomes smaller and smaller as it contracts from the wall of the container and the surface of the liquid. Milk that shows definite proteolysis has a bitter flavor due to protein decomposition products.

When litmus is present in the milk, coagulation commonly is preceded by reduction of the litmus that is complete except at the surface of the milk. With some cultures reduction is delayed and is not complete until after coagulation; from the standpoint of reduction of litmus, the slow reducing cultures are comparable to *S. lactis* var. *anoxyphilus*. Before proteolysis is evident, litmus milk cultures of *S. liquefaciens* appear essentially the same as cultures of *S. lactis*.

General characters. *S. liquefaciens* is a typical *Streptococcus*. In milk the cells are arranged singly, in pairs, and in short chains. The organism is Gram positive and does not form spores. Except for proteolysis of milk and liquefaction of gelatin, the general cultural characters are essentially the same as those of *S. lactis*.

The lactic acid produced is *d* or *d* and *l*. Acetylmethylcarbinol regularly is produced in milk and some cultures form relatively large amounts; the yield commonly is much greater at 21.1 C (70 F) than at 37 C (98.6 F). With certain cultures production of acetylmethylcarbinol is increased by addition of citric acid to the milk.

S. liquefaciens grows at 10 C (50 F), 45 C (113 F), and temperatures between; growth is much more rapid at 37 C than at 10 C. The cultures studied by Long and Hammer [47] varied somewhat in heat resistance. All of them survived 61.7 C (143 F) for 20 min, most of them survived for 50 min, but all of them were destroyed at 65.6 C (150 F) in 40 min. *S. liquefaciens* survives in cultures much longer than *S. lactis.*

Distribution and importance. *S. liquefaciens* is widely distributed in dairy products but in normal materials ordinarily is not present in large numbers. It has been most regularly isolated from cheddar cheese.[47] The organism is of little importance in production of acid in raw milk and cream, but in certain instances it apparently has been responsible for bitterness in pasteurized products.

Isolation. The proteolytic property of *S. liquefaciens* is an aid in its isolation. Materials can be plated on agar to which sterile milk has been added and, after incubation, actively proteolytic colonies that suggest streptococci can be picked into litmus milk and studied. When the organism is present only in small numbers there is an advantage in using heavily seeded plates, so that a relatively large amount of material can be examined, and incubating at 37 C (98.6 F) for 16 to 24 hr; with such incubation *S. liquefaciens* develops well, but other species ordinarily do not grow to such an extent that they interfere seriously with the picking of the desired colonies. In attempting to pick *S. liquefaciens* from plates, proteolytic micrococci are responsible for most of the failures.

SPECIES OF THE GENUS *LACTOBACILLUS*

The genus *Lactobacillus* includes a variety of organisms that fall into two natural groups, the heterofermentative organisms, which produce a variety of compounds from the sugar fermented, and the homofermentative organisms which produce principally lactic acid from sugar. The homofermentative organisms that attack lactose are of special significance in dairy products because of their wide distribution and activity under certain conditions. Their general action on milk is much like that of *S. lactis* and the striking difference between the two groups is in morphology. They sometimes are referred to as the rod-shaped lactic acid organisms. Certain of the organisms are very acid tolerant and are capable of growing in milk and other dairy products after growth of *S. lactis* has been stopped by the accumulated acid.

Presence of lactobacilli in dairy products. Lactobacilli commonly are present in dairy products. Following investigations of the organisms in Europe, Hastings and Hammer [48] reported their wide distribution in milk, butter, and cheese in the United States, and Heinemann and Hefferan [49] found them normally present in feces of various animals, in a variety of sour and aromatic foods, such as dill pickles, olives, and sauerkraut, in cattle feed, in normal saliva and gastric juice, in fermented milk, in market milk, and in soil, both manured and unmanured. Lactobacilli play little part in the beginning acid development in milk and cream because of the rapid growth of *S. lactis,* but if holding is extended may become active when the other organisms have produced their maximum acidities. In certain areas producing sour cream for buttermaking, the cream occasionally shows excessive acidities during the warm months, owing to the action of lactobacilli. Whey in vats or returned to the farms was found by Dotterrer and Breed [50] to contain many of these organisms. They also commonly are present in ripened cheeses, often in large numbers.

The homofermentative lactobacilli that attack lactose are well adapted to growth in dairy products generally. They are greatly favored by the lactose, and their acid tolerance permits their development under conditions which inhibit many bacterial species.

Species important in dairy products. Apparently, several species of homofermentative lactobacilli are important in dairy products. Their classification presents difficulties and what appears to be the same organism may be referred to by more than one name. In some cases a culture containing a *Lactobacillus* for use in cheesemaking has been referred to by a number because of the difficulty of identification.

The organism now known as *L. casei* apparently was first studied by von Freudenreich [51,52] in investigations on the ripening of swiss cheese. It is quite regularly present in milk in the United States and at least certain other countries; for example, Steuart [53] found lactobacilli invariably present in milk at Cardiff, Wales, and it is probable that they should be classed as *L. casei.* Sherman and Stark [54] noted that *L. casei* generally is present in milk in considerably larger numbers than either *L. acidophilus* or *L. bulgaricus.* With grade A milk, only 12 per cent of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers as great as 1 per milliliter, whereas 71 per cent contained *L. casei* in numbers as large; with ordinary milk, only 2.3 per cent of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers exceeding 10 per milliliter, whereas 94 per cent contained *L. casei* in excess of 10 per milliliter.

L. acidophilus was first isolated from the fecal material of infants by Moro [55] and now is recognized as more or less regularly present in the normal intestinal tract of man and other warm-blooded animals. Cultures of the organism are used in attempts to control the intestinal flora; it appears to colonize better than other lactobacilli.

L. bulgaricus originally was isolated by Grigoroff [56] from fermented milk. It received considerable attention as a result of the recommendation of Metchnikoff that it be used to control intestinal putrefaction which this investigator believed was an important factor in limiting the length of life in man. The suggestion was based on the theory that since putrefactive organisms are sensitive to acid their development in the intestinal tract can be controlled by organisms producing considerable acid. *L. bulgaricus* was selected by Metchnikoff because it produces a comparatively high acidity in milk and also because of the presence of this organism in the sour milk consumed in large quantities by certain races noted for their long life. However, extensive trials indicate that *L. bulgaricus* does not establish itself in the intestinal tract, even when consumed in large numbers.

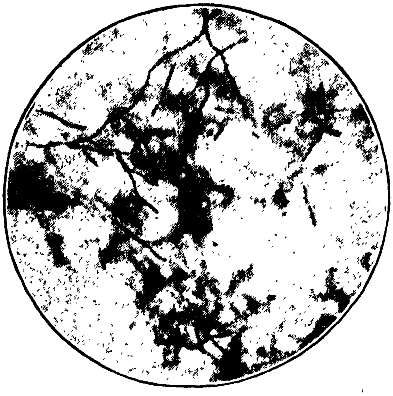

FIG. 6. *Lactobacillus acidophilus* showing chains.

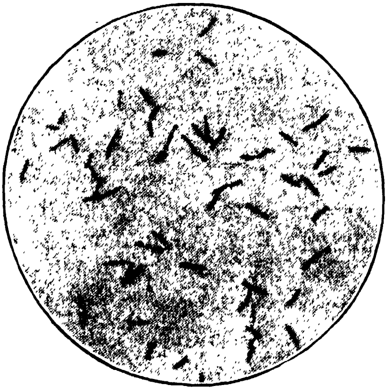

FIG. 7. *Lactobacillus bulgaricus* showing granules.

General description of *L. casei*, *L. acidophilus*, and *L. bulgaricus*.

Morphology. *L. casei*, *L. acidophilus*, and *L. bulgaricus* are definite rods that vary a great deal in size, with *L. bulgaricus* tending to be larger than the others. The cells may occur singly or in chains; some *L. bulgaricus* cultures show unusually long chains. The organisms stain readily and are Gram positive, although some negative cells often are noted, especially in old cultures. Occasionally, stained preparations show distinct granules. Spore formation does not occur and the organisms are non-motile.

Cultural characters. Growth is more rapid in milk than in any other material, and generally is poor on agar media; tomato, whey, and beef infusion agars are more satisfactory than nutrient agar. In litmus milk there ordinarily is coagulation and reduction of the litmus, except for a pink band at the top. Gas is not produced. Growth is definite, but slight, in bouillons containing various fermentable materials. Colonies are small and somewhat variable in type, and the edge may be either smooth or filamentous; some *L. bulgaricus* cultures give a very filamentous edge.

Biochemical features. Acid is produced from a number of materials but there is no great regularity in those fermented. In milk, acid develops readily at favorable temperatures. The final acidity varies a great deal, with some cultures giving only a slight coagulation in milk and others producing more than 4 per cent acid; *L. bulgaricus* yields especially large amounts. The isomeric form of lactic acid produced is variable and results obtained by different investigators for any one of the species are not constant. The percentage of the theoretical yield of lactic acid is high, up to approximately 98 to 99 per cent, and in accordance with this the cultures are lacking in odor. Proteolysis of the casein usually can be detected by chemical examination, although it is not suggested by appearance of the milk cultures.

FIG. 8. Lactobacilli in old, very acid cream.

Growth conditions. A comparatively high temperature, such as 32.2 to 37.8 C (90 to 100 F), greatly favors growth. *L. acidophilus* and *L. bulgaricus* grow at 45 C (113 F) but not at 15 C (59 F); *L. casei* does not grow at 45 C but does grow at 15 C and also at lower temperatures. The organisms are facultative.

Differentiation of homofermentative lactobacilli. The homofermentative lactobacilli that ferment lactose and are important in dairy products are differentiated only with difficulty. Various tests have been suggested for such differentiation but most of them show irregularities when applied to a series of cultures. There is evidence that the source of an organism should be taken into consideration in its classification.

Importance of lactobacilli in dairy products. Development of high acidity in cream intended for churning is very undesirable because of the influence on the flavor of the butter. The effect may be decreased by partial neutralization but it cannot be entirely controlled. In the ripening of certain cheeses, lactobacilli play an important role since they are one of the factors involved in the protein decomposition. Lactobacilli also are desirable in certain fermented milk preparations.

Sources of the lactobacilli. Trials at the Iowa Agricultural Experiment Station indicate that milk does not contain lactobacilli when it leaves the udder but that they are present by the time the milking is complete. The organisms regularly are found in the stable surroundings. Grains and various other feeds contain them. Hunter and Bushnell,[57] Sherman,[58] and Heinemann and Hixson[59] found large numbers in silage; Sherman reported that they constantly are present in considerable numbers on corn fodder so that corn silage always is amply seeded. The organisms are present in cow manure. Their presence in various feeds partly accounts for this, but multiplication in the intestinal tract also is a factor. Orcutt[60] found the organisms were present throughout the small intestine of young calves and were the predominating type in the fourth stomach.

Isolation of lactobacilli. Lactobacilli are not readily isolated because of the difficulty with which they grow on media adapted to plating. In isolating from milk a bottle should be filled, stoppered with a cork, and then held at a favorable temperature, such as 37 C (98.6 F), so that the desired organisms will increase in numbers. *S. lactis* and various other species will multiply rapidly at first, but when they are restrained by the acid developed the lactobacilli will multiply. Microscopic examinations make it possible to follow the fermentation. When the lactobacilli are present in large numbers, the milk can be plated on tomato, whey, or beef infusion agar, the plates incubated at 37 C, and colonies picked into litmus milk. Growth on agar frequently is very poor and colonies may not develop even when large numbers of organisms are present in the milk. In some instances shake cultures in tubes have yielded better colony development than plates, owing presumably to the oxygen requirements of the organisms. It sometimes is an advantage to transfer a loop of fermented milk from the first bottle to a bottle of sterile milk and allow this to develop acid, with the idea that the desired organisms will so dominate the fermentation that little difficulty will be experienced from other species. It is necessary to have the bottles practically full and stoppered tightly so that there will be little opportunity for growth of molds. Lactose-fermenting yeasts sometimes cause difficulty by blowing the stoppers.

Acid tolerance of the lactobacilli makes it possible to check other types by means of added acid. One of the procedures used is to inoculate the material from which the isolation is to be made into bouillon containing a fermentable material, such as dextrose, and enough acetic acid to give approximately an N/10 solution. Growth can be followed microscopically and if the lactobacilli develop satisfactorily plates can be poured. A series of transfers through the acid bouillon may yield essentially a pure culture of lactobacilli.

SPECIES OF THE GENUS *BACILLUS*

The genus *Bacillus* includes certain species that differ conspicuously from most of the organisms in the group by producing a smooth curd and considerable lactic acid when grown in milk; if litmus is present there commonly is a pronounced reduction so that the cultures have the general appearance of *S. lactis.* Coagulation is due to a rennin-like enzyme, since it occurs at acidities such as 0.30 to 0.40 per cent, but acid production continues until 0.50 to 0.90 per cent is present. The organisms require comparatively high temperatures for active growth and are not found in significant numbers in milk held under the usual conditions. Occasionally, they are encountered in milk and certain of its derivatives when the holding temperatures are so high that the species usually active cannot grow, or when the method of treatment was such that only spore-forming organisms survived. Organisms of this general type have been of the greatest practical significance in evaporated milk. Although the sterilization exposures used are intended to preserve the product, irregularities sometimes occur and one of the types of spoilage that results is coagulation and acid development.

Bacillus coagulans [61] illustrates the lactic-acid-producing organisms of the genus *Bacillus.* It grows well at 37 C (98.6 F) and 55 C (131 F), but poorly, if at all, at 20 C (68 F). The lactic acid produced in evaporated milk is the *d* form. Volatile acid also is formed and consists of acetic and propionic acids. Both the soluble and amino nitrogen are increased in milk. *Bacillus calidolactis* [62] is another organism of this general type. It grows rapidly at 55 C but does not grow at 37 C. In milk *d* lactic acid is produced; the amount of volatile acid formed is small.

It appears that acid production in milk as a result of growth of organisms of the genus *Bacillus* is becoming more common. Usually, the milk involved is being held at relatively high temperatures in connection with the preparation of concentrated products.

GASSY FERMENTATION

Development of gas in milk, cream, etc., through action of organisms constitutes the gassy fermentation. Composition of the gas evolved is variable, depending on the organism responsible, but carbon dioxide commonly is a prominent constituent. The organisms of most importance in producing gas in dairy products include (1) certain non-spore-forming yeasts that ferment lactose, (2) the *Escherichia-Aerobacter* group, and (3) certain organisms of the genus *Clostridium.*

Importance of gassy fermentation. In dairy products definite gas formation most often occurs in materials containing some acid. It is common in sour cream held for considerable periods, especially during the warm months. In the extreme condition the covers are blown from the cans when the wires attaching them are clipped, and the cream foams or boils from the containers. If the covers do not fit properly the cream may be forced out around the lids, owing to the vigorous evolution of gas. When the fermentation is less extensive, gas production is evident on stirring the cream because of the tendency to foam. Gassy cream often is referred to as "yeasty" because of the pronounced yeasty odor, and investigations of the condition by Hunter[63] and by Hammer and Cordes[64] show that the causative organisms are yeasts. With this fermentation there is a loss due to the cream that foams out of the cans and another as a result of the decreased quality of the butter that is the principal product made from sour cream.

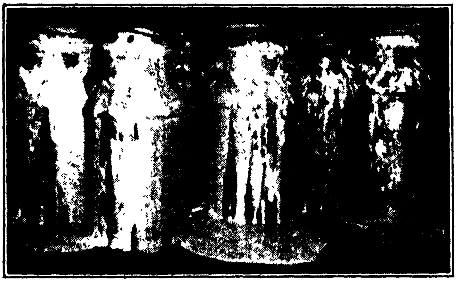

FIG. 9. Yeasty cream. Courtesy R. V. Hussong.

Development of gas sometimes occurs in milk being made into such products as cottage cheese and lactic acid culture. *Escherichia-Aerobacter* species are responsible in certain of these cases; yeasts are involved in others. Whey standing in vats or cans occasionally undergoes gassy fermentation which is most often due to yeasts; this change is favored by the relatively high temperatures at which whey commonly is allowed to stand.

LACTOSE-FERMENTING YEASTS

In the United States the yeasts of importance in the development of gas in dairy products include two species, *Torula cremoris* and *Torula* *sphaerica.*[64] The organisms produce carbon dioxide and small amounts of ethyl alcohol from lactose; because of their acid tolerance they can develop in materials in which the acidity prevents growth of many organisms. The striking differences between the two species are in shape and size of the cells and in growth temperatures. *T. cremoris* has an ellipsoidal shape and grows better at 37 C (98.6 F) than at lower temperatures, while with *T. sphaerica* most of the cells are essentially spherical and there often is little or no growth at 37 C, the optimum temperature being somewhat lower. Both organisms produce a vigorous gas evolution and a yeasty odor in litmus milk at favorable temperatures. Reddening of the litmus also occurs, and in old cultures there sometimes is coagulation, especially in the lower portions. The organisms grow well on agar and give a raised, smooth-edged, shiny, chalk-white growth, having a yeasty odor.

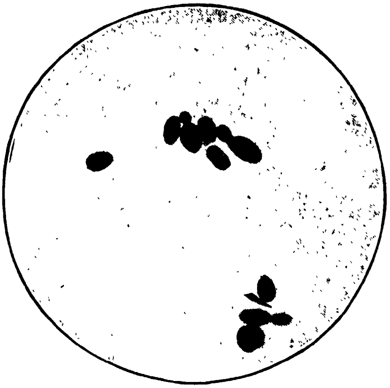

FIG. 10. *Torula cremoris.*

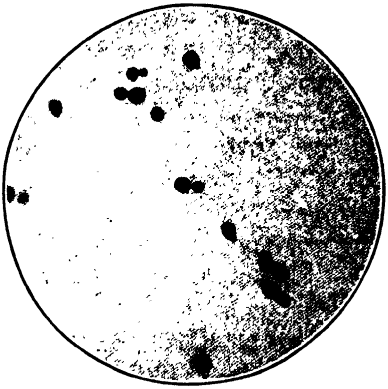

FIG. 11. *Torula sphaerica.*

Grimes and Doherty[65] consider that lactose-fermenting yeasts are of importance in the dairy industry because they produce undesirable changes in dairy products, and also because they interfere with the normal churning when cream is held for several days, as is done in home buttermaking. The investigators studied cultures isolated from milk, cream, and butter and recognized two species, ***Torula lactosa*** and *T. cremoris.*

Development of *T. cremoris* and *T. sphaerica* in cream. *T. cremoris* and *T. sphaerica* commonly are present in cream, either sweet or sour. Growth of the organisms is favored by comparatively high temperatures, and accordingly their rapid development is largely limited to the warm months. Occasionally, a lot of cream held in a heated room may show yeastiness during the winter, but the fermentation is decidedly a seasonal one and rather strictly confined to the summer. Acid tolerance of the organisms makes possible their development in sour cream.

In pure culture *T. cremoris* or *T. sphaerica* does not produce a typical foamy condition in milk or cream. Without coagulation there is little tendency for the gas to be held and it readily escapes. However, when coagulation has occurred, the gas tends to be retained and the volume of the fermenting material is increased by the gas bubbles present. In either sweet or sour cream there commonly is a supersaturation with gas and, when this comes out of solution on jarring or agitation, it readily escapes from uncoagulated cream but with the coagulated product it is partly held and yields a foamy mass. Accordingly, typical foaminess in cream is due to associative action, the yeasts producing the gas and some other species causing the necessary coagulation by development of acid.

Under practical conditions both *T. cremoris* and *T. sphaerica* are important in production of gas in sour cream and other acid materials; often a gassy sample contains both species.

Isolation of *T. cremoris* and *T. sphaerica*. One or both of the common lactose-fermenting yeasts often can be obtained from a sample of milk or cream by allowing it to stand at approximately 30 C (86 F) until the odor and gas production suggest there has been a good growth of the desired organisms, and then plating on some such medium as potato dextrose or whey agar. If sufficient tartaric or lactic acid is added to the medium to give a *p*H of 3.5, growth of bacteria is prevented and the isolation simplified.

ESCHERICHIA-AEROBACTER GROUP

The *Escherichia-Aerobacter* organisms ferment lactose with the production of gas and acid and, accordingly, are important in dairy products. They are Gram-negative, non-spore-forming rods that grow well aerobically. Gas production ordinarily is conspicuous in young milk cultures, and on being shaken they often develop a foam. Rate and extent of acid production vary a great deal, some cultures causing coagulation rather rapidly, others failing to coagulate even after long holding. When coagulation occurs the curd is not especially gassy because the gas largely escapes before precipitation of the casein takes place. The organisms do not proteolyze the casein in milk, although there sometimes is a collection of whey at the top of a culture that suggests this. Growth on the usual agar plates appears quickly and the mature colonies are large.

Biochemical characteristics. The biochemical characteristics of the *Escherichia-Aerobacter* organisms are important because of their extended growth in dairy products under certain conditions. The gas produced is primarily carbon dioxide and hydrogen, and the lactic acid is the *l* form. Lactose decomposition also yields other compounds, especially volatile acids.

Division of *Escherichia-Aerobacter* group. There are two primary divisions of the organisms included in the *Escherichia-Aerobacter* group: (1) those belonging to the genus *Escherichia,* of which *E. coli* is the type species, and (2) those belonging to the genus *Aerobacter,* of which *A. aerogenes* is the type species. An intermediate division also is recognized.

The genus *Escherichia* includes primarily the organisms coming from the intestinal tract of man and animals; the genus *Aerobacter* includes primarily those coming from soil and grains. Rogers *et al.*[66] called attention to the scarcity of the *Aerobacter* type in bovine feces. Later, these same investigators[67] pointed out that the *Escherichia-Aerobacter* organisms from grains do not agree with the characteristic flora of bovine feces. Kon[68] noted that among the *Escherichia-Aerobacter* organisms in bovine feces *E. coli* largely predominated, the proportion of *A. aerogenes* being small. Malcolm[69] isolated 342 *Escherichia-Aerobacter* cultures from 114 specimens of bovine feces and 96.4 per cent were *E. coli.* By use of enrichment methods *Aerobacter* organisms often were isolated from fecal samples so it appears these organisms are rather regularly present, but only in small numbers.

Presence of *Escherichia-Aerobacter* organisms in milk. Organisms of the *Escherichia-Aerobacter* group are practically always present in milk at the time it leaves the stable, although with very rigid methods of production relatively large volumes must be used to detect them. Ayers and Clemmer[70] found that milk produced under the worst conditions normally encountered rarely contained more than 2,000 of the organisms per milliliter. Finkelstein[71] reported that when care was used in the production of milk the number of *Escherichia-Aerobacter* organisms ordinarily was small, averaging less than 100 per milliliter; with indifferent methods the number averaged 588.

With 21,569 samples of milk taken under fair conditions of cleanliness Malcolm[72] found that 48.3 per cent showed no *Escherichia-Aerobacter* organisms in 0.1 ml, 21.4 per cent contained the organisms in 0.1 ml but not in 0.01 ml, 14 per cent contained the organisms in

0.01 ml but not in 0.001 ml, and 16.3 per cent contained the organisms in 0.001 ml. The proportion of positive samples was higher in summer and early autumn than in winter and spring, and there was a well-marked direct correlation between the proportion of samples containing *Escherichia-Aerobacter* organisms in 0.1 ml and the minimum and maximum atmospheric temperatures.

Yale and Eglinton[73] examined 882 samples of fresh raw milk obtained monthly from 85 producers and the *Escherichia-Aerobacter* counts were < 1 per milliliter with 46 per cent, < 10 per milliliter with 83 per cent, and < 100 per milliliter with 95 per cent; with 5 per cent the counts ranged from 100 to 5,500 per milliliter.

Data of various investigators show that milk commonly contains both *Escherichia* and *Aerobacter* species. Levine[74] summarized data from various sources and found that, of 1,382 cultures isolated from milk, 43.1 per cent belonged to the *Aerobacter* type. Hunter[75] reported that of 590 cultures obtained from milk, 66.2 per cent belonged to the *Escherichia* type, and of 169 cultures from bovine feces 95.2 per cent also belonged there.

Sources of the organisms in milk. Organisms of the *Escherichia-Aerobacter* group have occasionally been found in milk at the time it leaves the udder but this is unusual. They commonly get into milk through the general contamination with manure, soil, feeds of various types, etc. Hunter and Bushnell[57] found them in various kinds of silage studied. Ayers and Clemmer[70] noted that they also can come from unsterile utensils. In one series of trials in which utensils were allowed to contain milk drainings for 24 hr during extremely hot weather and then washed carelessly in lukewarm water, the *Escherichia-Aerobacter* count of the fresh milk averaged about 1,000,000 per milliliter; an attempt to duplicate the results was unsuccessful. Yale and Eglinton[73] reported the *Escherichia-Aerobacter* counts on fresh cow manure ranged from 100,000 to 10,000,000 per gram, and on dry cow manure they were as high as 90,000,000 per gram. At two dairies using milking machines, *Escherichia-Aerobacter* organisms were recovered in large numbers from the rubber parts at one dairy and from the milker pail at the other.

Growth of the organisms in milk. The *Escherichia-Aerobacter* organisms grow rapidly in milk when the temperature is suitable and multiplication may be the cause of excessive numbers. Ayers and Clemmer[70] reported that little or no growth occurs below 10 C (50 F). Finkelstein[71] also found that this temperature, or lower, checked the growth of the group in raw milk, and that a temperature of 15.6 C

(60 F), or higher, resulted in a rapid increase. Hunter[75] investigated the effect of temperature on the *Escherichia-Aerobacter* organisms in milk by taking 59 samples from an average dairy and making counts at once and after holding one portion at 15.6 C and the other at 21.1 C (70 F) for 24 hr. The fresh milk averaged 52 *Escherichia-Aerobacter* organisms per milliliter with variations from 1 to 1,000, that held at 15.6 C averaged 964 per milliliter with variations from 0 to 10,000, and that held at 21.1 C averaged 60,762,000 per milliliter with variations from 1,000 to 1,000,000,000. Many other investigators have shown that growth of *Escherichia-Aerobacter* organisms in dairy products often is rapid and that as a result definite defects are produced.

Importance in dairy products. The *Escherichia-Aerobacter* organisms always are undesirable in dairy products. In milk and cream, in addition to forming gas and acid, they produce objectionable flavors of a variety of types; when these products are used in the manufacture of cheese or butter the off flavors commonly are carried over, and unless the organisms are destroyed by pasteurization development of the off flavors may continue. Pont[76] reported that the *Aerobacter* and intermediate species were similar in their effects on cream flavor and produced a more serious and rapid deterioration than the *Escherichia* species. The organisms of the group may play a part in the natural souring of milk and cream. Heinemann[77] found them in large numbers in the initial stages of souring, whereas *S. lactis* dominated the field in the later stages. Certain strains of the *Escherichia-Aerobacter* organisms may produce ropiness in milk and cream. Yale[78] emphasized the variety of dairy products in which the organisms may cause defects.

In most of the studies on the *Escherichia-Aerobacter* organisms responsible for gas formation in dairy products the identification has not been carried to a species basis. However, the studies along this line show that various species are involved.

Sarles and Hammer[79] reported an unusual instance in which a few consumers of a raw milk supply complained that the milk seemed to be full of gas. A bottle of milk returned by a consumer had a normal appearance but on being shaken, or warmed, the milk foamed markedly. An *Aerobacter* organism caused the defect.

Isolation of *Escherichia-Aerobacter* organisms from dairy products. Organisms of the *Escherichia-Aerobacter* group can be isolated directly from dairy products by plating, either on standard agar or some differentiating medium, or an enrichment procedure, using bouillons of various types, may precede the plating.[80] Detection of the organisms in dairy products often is based on the development of gas in some selective medium.

SPECIES OF THE GENUS *CLOSTRIDIUM*

Certain of the spore-forming anaerobes of the genus *Clostridium* produce gas in milk or cream under favorable conditions, for example when the acid-producing organisms are destroyed by heat and the material held with a limited air supply. They are of little importance in raw milk or cream because of their inability to compete with the organisms normally present, but may be of significance in certain products. Their action occasionally is noted in evaporated milk that was understerilized. The organisms are easily demonstrated in many lots of milk by heating, for example at 80 C (176 F) for 10 min, and then holding under essentially anaerobic conditions. This procedure is the basis of a test for the presence of the general group of gas-producing, spore-forming anaerobes in small quantities of milk, such as 0.001 to 5 ml (page 43). Apparently, the organisms often come from fecal material and soil.

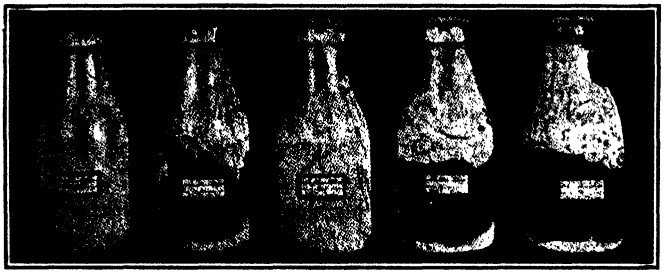

FIG. 12. Associative action in the appearance of gas in milk; bottle at left shows action of the gas former alone, while remaining bottles show action of the gas former in combination with *Streptococcus lactis*. Iowa Agr. Exp. Sta. Res. Bul. 27.

ROPY OR SLIMY FERMENTATION

In the ropy or slimy fermentation of milk, cream, etc., growth of organisms results in such a change in consistency that the materials adhere and form threads, or viscous masses, when poured or dipped. There is great variation in extent of the ropiness; sometimes the change is so slight that it is detected only by careful examination, and in other cases the material can be drawn into threads a yard or more in length and as fine as the threads of a spider web. In certain instances, particularly with some of the acid-producing bacteria, the ropy material is tough and doughy. If the ropiness is at all pronounced it is readily evident when the material is poured, or when a fork or spoon is drawn through it. In general, ropiness present in sweet milk or cream disappears as acid develops, whereas that accompanying a definite acid production frequently increases with an increase in acid and then later decreases. Vigorous agitation markedly decreases the ropiness. Normal cream with a high fat content occasionally shows a consistency which suggests a slightly ropy condition to anyone unfamiliar with the product.

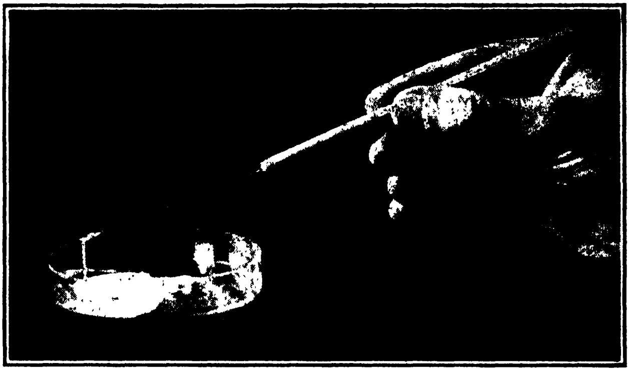

FIG. 13. Ropy cream. Causative organism *Alcaligenes viscosus.*

Mattick [81] reported formation of threads on the sides of a cooler over which milk was flowing. Some of the threads persisted in an apparently solid form for several minutes and extended over several corrugations. On removal from the cooler the threads at once assumed the liquid form and the milk collected from the cooler was indistinguishable from the uncooled milk. When allowed to flow under experimental conditions, a number of samples of milk from different sources, both mixed milk and that from individual cows, showed thread formation. The threads were considered to be due to the formation of thin films of casein and/or lactalbumin at the milk-air interface.

Ropy milk should be distinguished from gargety milk coming from certain abnormal udders. With gargety milk the unusual condition is due to the presence of masses of fibrin and leucocytes thrown out by the udder tissues in response to an infection and also to the abnormal composition; with ropy milk the consistency is due entirely to growth of bacteria in what originally was a normal product. Gargety milk shows the abnormal condition at the time it is drawn and, when not evident from the appearance of the milk itself, often is recognized by the material collecting on the strainer cloth or in the clarifier bowl, whereas ropiness ordinarily is not present until the milk has stood at least 12 hr.

Importance. Ropiness is one of the common abnormal fermentations of milk and its liquid derivatives. Undoubtedly, it is most frequently encountered and is most serious in market milk, where it causes much annoyance to consumers and dealers. Milk may be delivered in a normal condition and then show ropiness after being held by the consumer. This often occurs when the holding temperature is quite low because some of the organisms producing ropiness grow unusually well at fairly low temperatures and determine the change taking place there. At higher temperatures they are overgrown by the lactic acid organisms and the milk sours. Often only the cream is ropy and the milk beneath is normal; this is because of the oxygen requirements of certain of the causative organisms, although concentration of bacteria in the cream layer also may be a factor. Milk consumers frequently assume that ropiness is due to a diseased condition in the producing animals and, unless the dealer quickly brings the defect under control, loss of patronage may result. The organisms causing ropiness appear to be entirely harmless to persons using milk or cream in which they have grown.

Sweet cream delivered to dairy plants sometimes shows a ropy condition and, as with sweet milk, this is most likely to occur when the holding temperature is rather low. Ropiness occasionally is noted in sour cream and in lactic acid cultures; in these products it is usually due to action of the organisms involved in the acid development.

Organisms causing ropiness in market milk. One of the more common of the ropy milk organisms is *Alcaligenes viscosus* which probably was first isolated by Adametz from water. After noting the action of the organism in milk and predicting that it might be the cause of ropiness in this product, Adametz [82] obtained it from ropy milk. It has been isolated repeatedly in Europe and also has been encountered in many sections of the United States. In old cultures there is less acidity than there was in the original milk, but the reaction change occurs slowly. *Alc. viscosus* is one of the ropy milk organisms that definitely requires air and, accordingly, when it is the causative organism in outbreaks, the ropiness often is noted in the cream only; in pure cultures in skim milk there frequently is a membrane at the surface. The organism produces a large capsule that can be demonstrated best in very young cultures, since in older cultures the capsules from different cells unite and form long threads, leaving the cells free or embedded in the capsular material, instead of each having a definite capsule. *Alc. viscosus* grows well at fairly low temperatures, such as 10 C (50 F), although growth is faster at somewhat higher temperatures. At 37 C (98.6 F) growth occurs but ropiness in milk is not as pronounced as at lower temperatures.

Long and Hammer[83] reported that organisms which are like *Alc. viscosus,* except for the failure to produce ropiness in milk and other liquid media, are encountered among organisms isolated from dairy products. The designation *Alcaligenes viscosus* var. *dissimilis* was suggested for them. Some of the cultures which produced no ropiness when first isolated gave ropiness after a number of transfers in milk. Typical *Alc. viscosus* cultures varied widely in the extent of the ropiness produced and in the period required to bring about the change. The investigators noted that *Alc. viscosus* hydrolyzes fat. Buchanan and Hammer[84] studied an organism producing ropiness in milk which was essentially like *Alc. viscosus,* except that it eventually developed an acid reaction in milk.

Organisms of the *Escherichia-Aerobacter* group have caused many outbreaks of ropiness in the United States. Although most strains belonging to the group do not produce a ropy condition when grown in milk, certain of them do; organisms of the latter class occasionally are isolated from dairy products which show no evidence of ropiness. *Escherichia-Aerobacter* organisms produce a ropy condition in milk by development of capsular material that forms threads through the milk, as with *Alc. viscosus.* Although the organisms produce gas in milk, gas production usually is not noted in milk developing ropiness under practical conditions. Acid production of the organisms also appears to be of minor importance and milk is criticized for the ropiness, without any mention of acid. At the Iowa Agricultural Experiment Station an *Escherichia-Aerobacter* organism caused ropiness in cream with an acidity of only 0.16 per cent.

Escherichia-Aerobacter organisms causing ropiness in dairy products have been identified on a species basis in comparatively few instances but the available data indicate various species are involved.

In the *Escherichia-Aerobacter* group development of ropy cultures from non-ropy ones is indicated by the work of Stark and Stark,[85] who found that cultures of *A. aerogenes* could be dissociated into a slimy type which produced ropiness in sterile milk. Dissociation was most quickly induced by addition of an amount of bacteriophage too small to cause complete lysis of the culture. *A. aerogenes* bacteriophage was demonstrated on lettuce, carrots, corn silk, and other plant tissues.

Coccus forms have been responsible for ropy milk. Hammer and Cordes [86] reported an outbreak due to a coccus that they named *Staphylococcus cremoris-viscosi.* It rapidly digests milk but develops pronounced ropiness before the digestion is evident. O'Droma and Grimes [87] found *Micrococcus pituitoparus* the cause of ropiness in milk from farms and city vendors, particularly during the winter and spring months. In milk under favorable growth conditions, the organism seemed to crowd out and almost entirely inhibit development of the bacteria normally present. Ropiness did not appear to be dependent on development of capsules but probably was related to the peptonizing action of the organism on proteins, with resultant formation of mucins. *M. pituitoparus* was not commonly found in water but may have been carried into surface waters from plant and soil sources during periods of heavy rains. The organism was easily destroyed at 62.8 C (145 F) for 15 min or 76 C (168.8 F) flash.

Prouty [88] studied an outbreak of ropiness in commercially pasteurized milk and isolated a thermoduric *Micrococcus* closely related to *M. freudenreichii.* Contamination was traced to one of the farms supplying the milk for pasteurization. While a detailed study of the conditions prevailing on the producing farm was not made, control methods which were put into effect did not overcome the defect. Outstanding characteristic of the organism was its ability to rapidly produce ropiness accompanied by an abnormal odor and followed by active digestion of the curd. The organism survived 61.7 C (143 F) for 20 to 35 min. M'Kenzie *et al.*[89] studied ropiness in milk that was caused by an unidentified *Micrococcus* and found that dust from newly made hay, if allowed to come in contact with milk during the course of production, may cause ropiness.

Organisms causing ropiness and considerable acid. Ropiness frequently is associated with production of considerable acid. Lactic acid cultures may develop this condition after varying periods of successful propagation, and ropy sour cream occasionally is encountered. Certain cultures of *S. lactis* (*S. lactis* var. *hollandicus*), *L. casei,* and *L. bulgaricus* show ropiness and this property appears to be lost or acquired with these organisms for no apparent reason, although in general a culture maintains either a ropy or non-ropy character. The ropiness may be evident before any considerable acidity develops; often it increases for a period and then finally decreases as the acidity becomes pronounced. The ropiness is due to conditions other than capsule formation. *S. lactis* cultures frequently show some cells with capsules, but the percentage of encapsulated cells is no larger with ropy cultures than with non-ropy ones. *L. bulgaricus* apparently does not produce capsules; long chains of cells may tend to make the fermented milk draw out into threads, although rather long chains are present in some cultures that are not ropy. *L. casei* sometimes shows chains of cells but more often does not. It produces capsules in both ropy and non-ropy cultures. There is some evidence that the number of organisms per milliliter determines, to a large extent, the presence or absence of ropiness.

Ropiness in whey. Ropy whey, in which the ropiness was due to *S. lactis* var. *hollandicus,* was at one time added to the milk used in making edam cheese. A ropy condition due to *Alc. viscosus* was observed at the Iowa Agricultural Experiment Station in the whey collecting when curdled milk was cut in the manufacture of cottage cheese; such a relationship is unusual since this organism commonly is not conspicuous in the presence of considerable acid.

Ropiness due to associative action. Buchanan and Hammer[84] reported ropiness in cream that was due to associative action of two organisms, one of which was *S. lactis.* The other organism produced no change in milk in 3 or 4 days, but when grown with certain *S. lactis* cultures ropiness quickly developed. Ropiness produced by the combination appeared to be due to a large number of organisms since the plate method and microscopic observation showed that the mixed cultures contained many more organisms than pure cultures of either species. The mixed cultures in milk also gave a slightly lower acidity than pure cultures of *S. lactis,* and this may explain the greater multiplication in the mixtures.

Ropy organisms of no practical importance. Many organisms from a variety of sources have been described as producing ropiness in milk but most of them have not caused outbreaks and probably never will be of significance since they are so readily overgrown by other species. From the available data it appears that in the United States *Alc. viscosus* and *Escherichia-Aerobacter* organisms are the important species from the standpoint of economic losses due to ropy milk.

Materials causing ropiness. The materials responsible for the ropiness developed in milk by certain bacteria apparently are of two types, the true gums or gum-like substances and the mucins. The gums are polysaccharids; the mucins are nitrogenous mucus-like substances.

It is probable that gums are more commonly the cause of ropiness in milk than mucins; with certain organisms the gums produced appear to be galactans.

Sources of ropy milk organisms. In a ropy milk outbreak the source of the responsible organism is an important consideration. After the organism once is established, infection undoubtedly occurs from the utensils and equipment, stable, coats of the animals, etc., but control measures give only temporary relief unless the original source is controlled.

Extensive studies on the flora of aseptically drawn milk show that ropy milk organisms ordinarily are not present in the udder. However, organisms capable of producing ropiness in milk have been obtained from this source and in an outbreak caused by *Staph. cremoris-viscosi* [86] the organism was found in milk drawn so as to exclude outside contamination, although it was impossible to determine whether the udder invasion preceded or followed the outbreak.

In outbreaks of ropy milk, the organisms often come from surface water. One of the common routes of infection is contamination of the coats of the cows from which the organisms then fall into the pail at milking. Harding and Prucha [90] reported that in two instances in connection with an outbreak studied by them material collected from the flanks and udders of cows produced characteristic ropiness when added to sterile milk. In some cases outbreaks of ropy milk have promptly followed flooding of the land on which the producing animals were pastured, and outbreaks have been stopped simply by keeping the animals from surface water. Isolation of *Alc. viscosus* from water before it was obtained from ropy milk is of significance in this connection. In addition to water standing in pastures, water from troughs, milk cooling tanks, and directly from wells must be considered.

When ropy milk organisms get into utensils they may live over from one milking to the next with the cleaning methods practiced on many farms. Harding and Prucha [90] noted that samples collected on farms delivering milk containing ropy organisms showed the organisms were in the utensils; at one farm the organisms were repeatedly isolated from utensils and from water in the cooling tank. Ward [91] found *Alc. viscosus* the cause of ropiness in two creameries in both of which the organism appeared to come from insufficiently scalded utensils. Greater care in scalding brought the trouble to an end. In one outbreak three different lots of sterile milk picked up the organism from the surface of a strainer.

Stark and Foter [92] obtained results indicating cow feeds are important sources of ropy milk organisms, since alfalfa hay, soy bean meal, and cottonseed meal contained many thousands of them per gram; certain other feeds did not yield the organisms.

Controlling ropiness. In combating an outbreak of ropy milk sterilization of all utensils and equipment coming in contact with the milk, including bottles and cans, is one of the first steps. The cooling tank may be a source of organisms through water splashing into the milk. Stables should be given a thorough cleaning and disinfection, since in some instances the milk apparently is contaminated from the air of stables. Flanks and udders of the cows can be wiped with a cloth dampened with disinfecting solution, and continued contamination of the coats of the animals prevented by keeping them from surface water. Control of an outbreak often is difficult and there may be a recurrence when the precautions taken are neglected, even for a short period. In certain cases ropy milk has been eliminated only after a long period of attention to many details. Pasteurization of the milk usually gives immediate relief, but outbreaks also occur in pasteurized supplies; with smaller dealers not regularly pasteurizing, use of the process commonly makes it possible to prevent ropiness immediately and thus retain customers that would be lost if the ropiness continued.

Heat resistance of common ropy milk organisms. Harding and Prucha [93] investigated the heat resistance of cultures isolated from outbreaks of ropy milk, including both *Escherichia-Aerobacter* organisms and *Alc. viscosus*. When heated in bouillon, some of them survived 60 C (140 F) for 10 min but not 65 C (149 F) for this period. In ropy milk a number of cells survived 60 C for 20 min but not for 30 min.

In a consideration of the influence of age of the bacterial cell on its heat resistance Sherman *et al.*[94] reported that some important milk bacteria (for example, ropy milk types) may be entirely eliminated from milk by pasteurization when the cells are in a young and growing condition, whereas old cells of the same organisms are able to withstand the process.

Hammer and Hussong [95] studied the heat resistance of *Alc. viscosus* from three sources: (1) an outbreak of ropiness in pasteurized milk, (2) butter which showed ropy moisture droplets at the surface and which was made from pasteurized cream, and (3) ropy whey obtained from pasteurized milk set for cheese. Cultures of various ages failed to survive 61.1 C (142 F) for 3 min. Three strains of *A. aerogenes* that produced ropiness in milk varied greatly in their heat resistance. With one strain organisms from an old milk or agar culture survived 62.8 C

(145 F) for 10 min but those from young milk cultures did not; with a second strain organisms from young or old milk or agar cultures failed to survive 61.1 C for 3 min; with a third strain organisms from an old milk or agar culture regularly resisted 62.8 C for 10 min and, in some instances, for 20 min although organisms from young cultures failed to resist these exposures.

Ropiness in pasteurized milk. Extensive outbreaks of ropiness occur in pasteurized milk. In general, they are due to contamination from plant equipment after the heating, especially from equipment that is difficult to sterilize properly, although some irregularity in the plant operations also may be responsible. Outbreaks in pasteurized milk can be stopped by thorough cleaning and disinfection of the plant and equipment, but recurrence frequently is noted. The presence of ropy milk bacteria in the raw milk coming to the plant is a possible source of the organisms.

In investigating an outbreak that had continued more than 2 years, Kelly[96] found the ropiness was due to organisms gaining entrance to the milk through pipes that were used first for raw and then for pasteurized milk. The difficulty was aggravated by bringing into the plant milk that was returned from stores and wagons; one lot of returned milk contained 32,000 ropy milk bacteria per milliliter. When the returned milk was kept out of the plant and other faults corrected, it was possible to eliminate the organisms from the equipment by use of steam, hot water, and chlorine.

Isolation of the causative organism. Isolation of the causative organism from ropy milk or cream usually can be accomplished by plating when the defect is due to such organisms as *Alc. viscosus* or an *Escherichia-Aerobacter* species. With these types, the viscous character of the colonies makes them easily detected in a mixed flora. Some of the plates poured should be incubated at 21.1 C (70 F) since with *Alc. viscosus* and *Escherichia-Aerobacter* species the colonies often are less ropy at 37 C (98.6 F) than at lower temperatures. If the ropiness may be due to *S. lactis* var. *hollandicus* or a *Lactobacillus* it is advisable to plate on tomato or whey agar in addition to standard or beef infusion agar and to pick colonies into sterile milk and incubate at 21.1 C to detect the ropiness.

When samples of normal milk are to be examined for ropy milk organisms it is advisable to hold them at relatively low temperatures, such as 10 C (50 F), and observe for ropiness after various holding periods. At these temperatures certain ropy milk organisms grow more rapidly than most of the other species present and thus are easily detected.

Distribution of ropy milk organisms. The common presence of ropy milk organisms in milk, even when ropiness does not develop, was shown by Ward [97] who found that the milk furnished by 42 patrons out of a total of 307 delivering milk to three plants became ropy when held 4 days at 4.4 to 7.2 C (40 to 45 F); no complaints of ropy milk were received at the plants. The investigator noted that in several plants ropy milk organisms frequently were present in any utensil that had been washed but not scalded, and in various pieces of equipment. Harding and Prucha [93] reported that holding individual samples from 140 farms supplying a bottling plant having trouble with ropy milk yielded ropiness one or more times with the milk coming from 116 of the farms. Kelly [96] examined milk from farms and found 17 of 150 samples contained organisms producing ropiness. A more detailed study of milk from 31 farms showed that milk from 6 became ropy in 2 days at 14.4 C (58 F).

Organisms which produce ropiness in milk often are isolated from milk or cream which either is normal or shows abnormal conditions other than ropiness. It is evident that ropy milk organisms can be present in these products without causing a change in consistency; this is due to their overgrowth by other types.

Use of ropy milk. Ropy milk resulting from the growth of *L. bulgaricus* has been added to buttermilk that "wheys off" rapidly to delay settling of the casein. Some of the fermented milk consumed in certain areas, particularly the Scandinavian countries, is ropy and, while it is not prized because of the ropiness, the condition is not objectionable. Macy [98] isolated a *Streptococcus* producing ropiness from a ropy milk beverage used by certain Finns in the United States.

SWEET CURDLING FERMENTATION

The sweet curdling fermentation is the process by which organisms curdle milk or cream through production of a coagulating enzyme comparable to rennin. Milk or cream that curdles at ordinary temperatures with an acidity too low to account for the coagulation ordinarily has been changed by such an enzyme. Some sweet curdling organisms produce little or no acid but others form considerable amounts after the curdling has taken place so that in detecting sweet curdling the acidity should be determined as soon as coagulation occurs.

Importance of sweet curdling. Sweet curdling is one of the more uncommon milk fermentations although it is by no means rare. Milk and cream delivered to dairy plants occasionally show this change, and it sometimes is observed by the retail purchaser of these products, especially when they are held for considerable periods after pasteurization. Sweet curdling is encountered most frequently during the warm months, or when the product involved is held at a fairly high temperature.

Cream with a normal acidity occasionally coagulates during pasteurization in butter plants and this has been assumed to be due to the presence of small amounts of coagulating enzyme whose activity is increased at comparatively high temperatures. The work of Frazier [99] confirms this idea. He found that although small amounts of milk culture of a sweet curdling organism inhibit heat coagulation over a small range, large amounts hasten it. When purified bacterial rennin was used there was no influence on the heat coagulation if the milk was heated immediately upon addition of the enzyme, but the heat coagulability of the milk was increased if the rennin was allowed to act for a time. The optimum temperature for most rennins was found to be 40 to 43 C (104 to 109.4 F), whereas 60 C (140 F) for 15 min destroyed them.

Although the sweet curdling fermentation is not common under practical conditions, almost any lot of milk contains organisms capable of causing this change. Their failure to develop ordinarily is due to overgrowth by the lactic acid bacteria, and it requires unusually favorable conditions, with perhaps few competing organisms, for sweet curdling to occur.

Causative organisms. The organisms capable of sweet curdling milk are extremely varied in their characteristics and belong to widely separated genera; the general types are:

1. Aerobic spore-producing bacteria. The aerobic spore-producing bacteria include various species that are pronounced sweet curdlers. Organisms of this type regularly are present in milk and cream and can be isolated by heating to destroy the non-spore formers, holding under aerobic conditions at a temperature favorable for growth, such as 25 to 37 C (77 to 98.6 F), until curdling occurs, and then plating. In general, these organisms enter milk from dust, dry manure, etc., the spores enabling them to resist the desiccation to which such materials are subjected. The aerobic spore producers occasionally cause sweet curdling under practical conditions, especially in pasteurized milk or cream held at comparatively high temperatures.

Lawrence and Ford [100] studied the aerobic spore formers in the Baltimore milk supply and reported that they believed *Bacillus albolactis* to be the common cause of the clotting and peptonization occasionally seen with boiled milk, and also that it undoubtedly is a contributing factor in the changes seen in milk heated to temperatures such as 60 to 65 C (140 to 149 F). This organism was encountered less frequently in samples of milk heated with various exposures than either *Bacillus cereus* or *Bacillus subtilis.*

The aerobic spore formers involved in the sweet curdling of milk and cream present difficulties from the standpoint of classification.[101] Ordinarily, classification on a species basis is not necessary in dealing with the organisms in these products

Such lactic acid organisms as *B. coagulans* and *B. calidolactis* sweet-curdle milk before any considerable quantity of acid is formed.

2. *Non-spore-producing rods.* A number of non-spore-producing rods are capable of sweet curdling milk. With most of them the curd formed is very soft and inconspicuous and the striking thing is proteolysis. Organisms belonging to the genus *Proteus* are representative of this general group and illustrate the usual type of change. The non-spore-producing rods that cause sweet curdling often are encountered in studying the bacterial flora of milk and cream and, apparently, come from a variety of sources; they are rarely, if ever, responsible for sweet curdling under practical conditions.

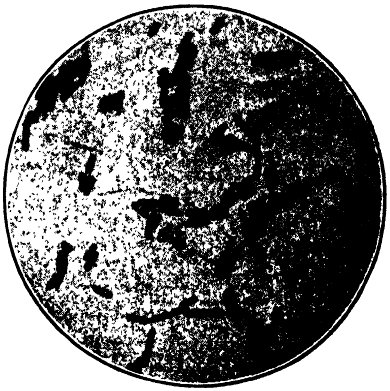

FIG. 14. Spore-forming sweet curdler.

3. *Coccus forms.* Various species of cocci, with widely differing characters, sweet-curdle milk. One of the more important is the lactic acid organism *S. liquefaciens* that sweet-curdles milk in which it is growing before the acid production is conspicuous. This organism has been found responsible for sweet curdling in a number of instances at the Iowa Agricultural Experiment Station; curdling usually was accompanied by a bitter taste.

Some of the micrococci sweet-curdle milk but bring about the change rather slowly and, accordingly, are unimportant as a cause of the fermentation under practical conditions.

4. Yeasts and molds. Various yeasts and molds can sweet-curdle milk but do it so slowly that they are of no practical significance.

PROTEOLYSIS

Proteolysis is the process by which casein, or some insoluble casein derivative, is broken down to water-soluble compounds through the action of organisms. Extent of the decomposition varies greatly with different species; sometimes it is almost complete and is readily evident, and in other instances it is slight and can be definitely established only by chemical determination of the amount of soluble nitrogen present. The decomposition products formed usually are a mixture of various groups of compounds and include peptones and related bodies, amino acids, ammonia, and, occasionally, free nitrogen.

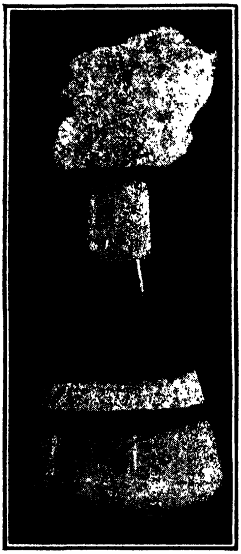

Fig. 15. Proteolysis following sweet curdling. The undigested surface portion is cream.

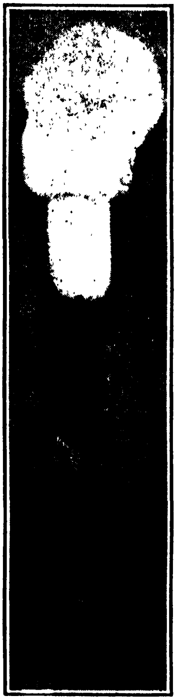

Fig. 16. *Streptococcus liquefaciens* in milk, showing proteolysis following coagulation.

Relationship of proteolysis and sweet curdling. The soluble nitrogen in milk is increased by various typical lactic acid species, but the organisms causing conspicuous proteolysis are sweet curdling forms and pronounced proteolysis rather regularly follows sweet curdling. It appears that, with certain organisms at least, the incubation temperature determines whether proteolysis or sweet curdling is the more evident. Sarkaria and Hammer [102] noted that with the organisms studied by them coagulation was more conspicuous at 37 C (98.6 F) than at room temperature. Observations of various investigators, including those of Frazier,[99] suggest that some of the organisms which sweet-curdle milk when first isolated, lose this property on cultivation and then have increased proteolytic powers. However, the effect of various salts on the activity of bacterial rennin, as pointed out by Frazier, may be a factor tending to cause variations in the results obtained with an organism.

The frequency with which proteolysis and sweet curdling of milk are associated in the characters of organisms has led to the suggestion that they always occur together and that, occasionally, one of the changes is overlooked in studying an organism because it is not pronounced at the temperature used. Cultures that have been described as proteolyzing milk without any mention of sweet curdling sometimes show a definite and complete sweet curdling when grown at suitable temperatures.

The proteolyzing action of certain organisms is evident on plates poured with media containing milk or casein. In some cases organisms that actively proteolyze on plates have only limited action when inoculated into milk. This may be due to the abundance of sugar in the milk which the organisms use in preference to the casein.

Bitterness following proteolysis. Proteolysis of milk and cream often is accompanied by development of a bitter flavor. This is due to the presence among the protein decomposition products of such bodies as peptones that are bitter. Sometimes bitterness is evident in milk or cream before either proteolysis or sweet curdling is conspicuous. Under practical conditions this occurs most frequently when the products are held at relatively low temperatures, such as 10 C (50 F), and *S. liquefaciens* often is the causative organism.

LIPOLYSIS

Various organisms hydrolyze fat with the liberation of fatty acids and glycerol, and the change is referred to as lipolysis. The lower fatty acids in milk fat, especially butyric and caproic acids, have conspicuous odors that are very objectionable in most dairy products. When the odors of the lower fatty acids develop in milk, cream, butter, etc., the condition commonly is referred to as rancidity. Lipolysis due to organisms should be definitely distinguished from that caused by the enzyme lipase which normally is present in milk.

Methods for detecting lipolytic organisms by means of differential plating media are given in Chapter 1. Using the same general procedures, pure cultures of organisms can be tested for their lipolytic powers by inoculating them on plates containing dispersed fat; observations are made directly, or after flooding the plates with an indicator, such as nile blue sulfate. An organism also can be tested for lipolytic action in other ways. It can be inoculated into sterile cream and the cream examined from time to time for the odor of the lower fatty acids, or it can be inoculated into sterile or pasteurized cream, the cream churned, and the butter examined at frequent intervals. The butter should be left in an unsalted condition since many organisms are inhibited by salt, and it should not be incubated above 21.1 C (70 F) in order to avoid seriously influencing the physical condition of the butter.

Some lipolytic organisms apparently assimilate certain of the products of fat hydrolysis and in this way the accumulation of various compounds may be delayed. The lower fatty acids are rather toxic for many species, especially in such a product as butter, and sometimes cause a rather rapid decrease in numbers of organisms.

Various organisms can hydrolyze fat and certain molds are especially active. Collins and Hammer[103] isolated 159 lipolytic bacterial cultures from different sources, such as water, air, dairy plant equipment, and dairy products, and found a variety of species. Common lipolytic bacteria are *Pseudomonas fragi,*[104] *Achromobacter lipolyticum,*[105] and *Pseudomonas fluorescens.* Many of the actively lipolytic organisms also are proteolytic.

Anderson[106] studied an organism characterized by its powerful fat-splitting action, weak action on protein, inability to act on sugars, and rapid growth at refrigeration temperatures. Refrigerated cream containing the organism liberated caproic, iso-caproic, and caprylic acids from the milk fat and they caused a peculiar irritation when the cream was swallowed. Rate of growth of the organism in milk at 4 C (39.2 F) was such that 1,500 bacteria per milliliter after 11 hr could become 1,000,000 after 60 hr and 130,000,000 after 110 hr. The organism now is designated *Achromobacter lipidis.*[107]

In milk and cream lipolysis due to bacteria is most commonly noted when the products are held at relatively low temperatures, such as 7.2 C (45 F). Under these conditions the lactic acid organisms are greatly inhibited and certain lipolytic species, especially *Ps. fragi,* develop relatively rapidly because of their psychrophilic character. Rancidity due to *Ps. fragi* commonly is preceded by an ester-like odor.

Lipolytic bacteria are most easily isolated by holding raw milk or cream at a rather low temperature until rancidity develops, and then plating on a differential medium.

OXIDASE PRODUCTION

Production of oxidase by micro-organisms is detected by color changes in the colonies when treated with such indicators as *p*-aminodimethylaniline hydrochloride and tetramethyl-*p*-phenylenediamine hydrochloride (page 22). Bacteria vary widely in their oxidizing powers and some of the strongly oxidizing types produce objectionable changes in certain dairy products. Castell and Garrard [108] found that *Pseudomonas* and *Achromobacter* organisms are the most strongly oxidizing types; *Alcaligenes* and *Brucella* organisms are somewhat less but still strongly positive; *Aerobacter, Escherichia,* and *Proteus* organisms are weakly positive, variable, or negative; *Bacillus* organisms are weakly positive or negative; cocci and the one anaerobe studied were definitely negative. All strong oxidizers are Gram-negative and definitely not strong oxidizers are Gram-positive. The authors noted that the organisms which produce the more serious defects in flavor of butter include almost wholly the strongly oxidase-positive bacteria; in all cases of surface taint, cheesy flavor, limburger flavor, or rancidity encountered, oxidase-positive bacteria were abnormally numerous.

In milk the oxidase-producing bacteria apparently do not produce oxidized flavor. Cone and Babcock [109] inoculated nine oxidase-producing bacteria into freshly pasteurized milk which was susceptible to development of oxidized flavor, either spontaneously or with addition of no more than 0.1 ppm copper. Amount of inoculation varied from < 100 to several million organisms per milliliter. None of the inoculated milk developed a greater intensity of oxidized flavor than the control when held 4 days at 4 to 6 C (39.2 to 42.8 F); with large inoculations development of oxidized flavor was markedly or completely inhibited. There was no evidence that oxidase-producing bacteria differ greatly with respect to oxidized flavor in milk from other bacteria common in milk. The nine organisms tested probably represented seven species, three of the genus *Pseudomonas* and the others belonging to the genus *Alcaligenes* or the genus *Achromobacter*.

FLAVOR FERMENTATIONS

Many changes in the flavor of milk and cream are brought about through action of organisms. Most species that grow extensively in these products definitely affect the flavor, although the influence is much greater with some organisms than with others.

Flavor includes the impressions obtained through the senses of taste and smell. These senses are so interrelated that impressions obtained through the sense of taste ordinarily are not differentiated from those obtained through the sense of smell, and what often is taken to be the taste of a product is in reality a combination of taste and odor.

Importance of flavor fermentations. Changes in the flavor of milk and cream through bacterial action are very common. Milk coming to market milk plants, cheese factories, and condenseries usually is examined for odors and/or tastes, many of which are bacterial, and certain butter factories have very high standards for their raw material. In products held for considerable periods at relatively high temperatures, there frequently are pronounced flavors due to organisms.

Abnormal flavors not due to organisms. Various factors other than activity of organisms may be responsible for abnormal flavors in milk and cream. These are of importance in a consideration of the flavor fermentations since it is necessary to know the cause of an abnormal condition before an intelligent attempt at its correction can be made. The following grouping includes most of the causes of abnormal flavors in milk and cream other than organisms.

1. Absorption of odors. Odors are quickly absorbed from the surroundings by exposed milk and cream, and this absorption may influence the flavor. Absorption is a common cause of such flavors as gasoline, fly-spray, disinfectant, cellar, etc., and may play a part with others, such as feedy and barny flavors. At one time it commonly was believed that milk would not absorb odors when it was warmer than the surrounding atmosphere, but it now is generally recognized that absorption occurs whether the milk is warm or cold. The rapidity with which milk can absorb odors is shown by the investigations of Russell;[110] horse manure was absorbed in 0.25 hr, cow urine in 0.5 hr, and silage in 1 hr. The fat is the constituent mainly involved in flavor absorption. Trout and McMillan[111] found that raw and pasteurized milk seemed to absorb odors slightly more than homogenized and heated milk wherein creaming was inhibited. Absorption was slightly greater when the fat was in the liquid state but also occurred when the fat was in the solid state. Absorption appeared to be primarily a surface problem, the absorbed odor being confined largely to the upper 10 per cent of a 10-gal can. When this layer was thoroughly mixed with the under layers the odor of the substance to which the milk was exposed could not be identified.

2. Materials consumed by the cows. Materials consumed by the producing animals may play an important part in determining the flavor of milk and cream. Feed flavors are imparted to the milk through the bodies of the cows and many feeds, such as silage, alfalfa, sweet clover, turnips, garlic, various weeds, etc., are responsible for definite abnormal conditions. Drugs and drinking water having pronounced flavors may act in the same way (Babcock [112]).

After a feed is consumed by a cow, a certain period is required before the flavor is evident in the milk. The flavor then increases in intensity for a time, later decreases, and finally disappears. Roadhouse and Henderson [113] noted that when the juice expressed from 25 lb of green alfalfa was administered as a drench to each of four cows, feed flavor was detected in the milk 20 min later. The flavor was most pronounced in the milk drawn 45 min after administering the juice and was less noticeable in the milk drawn after 2 hr. Babcock [114] reported detection of a garlic flavor in milk when the samples were taken 1 min after feeding garlic; intensity of the garlic flavor increased as the interval between feeding the garlic and milking increased, until after 10 min it was very pronounced. Strong garlic flavor was found in milk drawn 2 min after the animals had inhaled the odor of garlic for 10 min. Garlic odor was evident in samples of blood drawn after the feeding of garlic.

Certain feed flavors are largely seasonal. Garlic flavor is most common in the spring because garlic is one of the first plants to start growth and the animals then eat it readily; but it may occur at other times. Certain weed flavors are more frequently encountered in the late summer when the pastures are dry and the cows eat plants that were rejected earlier, when feed was abundant. Feed flavors often are more pronounced in cream than in the milk from which it came, owing to the absorbing power of the fat. Effect of feed on the flavor of milk sometimes is very evident when the cows are kept on one type of pasture during the day and another during the night. Flavors in milk due to the materials consumed by the producing animals represent instances of absorption before the milk leaves the udder. The constituents responsible for the flavors probably are carried to the udder through the blood and lymph.

3. Condition of the cows. Conditions in the producing animals may affect the flavor of the milk. When a cow is advanced in lactation the milk frequently shows an undesirable flavor that commonly is described as rancid, bitter, or salty, and an abnormal flavor may be present in milk from infected udders. With both these conditions there is great variation among different animals in the extent of the undesirable flavor, and it may even be entirely lacking.

The flavor present in milk as a result of advanced lactation often is not noticed until the milk has stood for a period. After it is evident it usually increases with the age of the milk. Eckles and Shaw[115] reported the flavor of the milk of four animals advanced in lactation as rancid and bitter combined, and stated that it developed within 24 hr. Palmer[116] found this abnormal flavor due to the activity of lipase which hydrolyzes the fat with liberation of fatty acids; the lower volatile fatty acids, especially butyric acid, largely cause the objectionable flavor. The enzyme is active, even at fairly low temperatures, but can be effectively retarded by pasteurization. Various factors influence its activity and these have been extensively studied (Chapter 7) because of the importance of the enzyme in various dairy plant operations. In advanced lactation there often is a high chloride content that may, in part, be responsible for the abnormal flavor, since this frequently is described as salty.

The off flavor in the milk of animals with infected udders presumably is due to variations in the composition, which occur as a result of the condition of the udder tissues.

Individuality of the animal also may be of importance from the standpoint of the flavor of the milk, entirely aside from such factors as feed consumed, stage of lactation, etc. Certain cows, even under very favorable conditions, give milk with more or less of an unpleasant flavor, and this may continue through succeeding lactation periods. It appears that with some animals the flavor due to advanced lactation occurs very much earlier in the lactation period than with others. Conversely, the milk of certain cows has a particularly pleasing flavor. Although this may be due in part to its richness, other factors also are involved. There may be differences in the flavor of the milk from the various quarters, and also from various portions of the milking. The fore milk sometimes has a rather pronounced unpleasant flavor and the strippings may have either a less pleasant or a more pleasant flavor than the main portion of the milking, depending undoubtedly on whether the off flavor is one associated with the fat.

Flavors due to the conditon of a producing animal are less often noted in the milk from herds than in the milk from individual cows since with the herd milk an off flavor in the milk of any one animal is more or less obscured by the mixing of the milk. However, these flavors sometimes involve considerable volumes of milk and cream.

4. Chemical action. When milk or cream is exposed to sunlight, it rather quickly acquires an off flavor which, when well developed, suggests tallow and often is referred to as tallowy. The condition is most often encountered in bottled milk left on the porch for some time after delivery. Hammer and Cordes[117] noted an off flavor in milk exposed to sunlight for only 10 min, and definite tallowiness was observed with exposures as short as 45 min. Exposed milk had a chalky appearance, and when churned yielded a lighter colored fat than the unexposed milk. Tallowiness develops on exposure to sunlight at very low temperatures and has been noted at the Iowa Agricultural Experiment Station in ice cream held at such a low temperature that it remained firm. Frazier[118] reported an off flavor in whole milk which had been exposed to diffuse daylight for 8 or more hours at freezing temperature. The light apparently acted as a catalyst in the oxidation of milk fat. The defect developed more rapidly in pasteurized than in raw milk.

A flavor that is referred to as oxidized flavor sometimes is present in milk held with minimum exposure to light. It appears most often in pasteurized milk at certain seasons, but also is encountered in raw milk. Development of the flavor is influenced by various factors such as presence of dissolved metal, particularly copper, feed of the animal, individuality of the animal, etc. The flavor appears to be due to an oxidation of one or more constituents of the milk and probably is related to the off flavor caused by light.

5. *Addition of foreign materials.* Foreign materials occasionally are added to milk unintentionally and the flavor influenced in this way. Soap may be left in a utensil; a pail or can may be employed in transporting oil, kerosene, or gasoline and then used for milk; or material on the hands of the milker or the udder may be washed into the milk.

Flavors produced by organisms. Flavors produced in milk through action of organisms are extremely varied. An acid flavor is the most common but many others are frequent. Flavors, such as feedy or barny, that suggest other causes may be due to organisms. In an instance studied at the Iowa Agricultural Experiment Station milk delivered by a certain producer frequently showed a flavor that was thought to be due to feed, but which investigation proved was the result of growth of an organism; the flavor was common in the night milk that was held for a period on the producing farm but was absent in the morning milk that was delivered soon after milking. Barny and other objectionable flavors apparently may be due to growth in milk and cream of the organisms which are responsible for decompositions in manure. Such specific flavors as soapy and turnipy occasionally are due to activity of organisms, instead of to the expected causes.

A malty flavor often is present in milk and cream as a result of growth of *S. lactis* var. *maltigenes*[119] and may be regularly noted in products from certain farms. *Escherichia-Aerobacter* organisms frequently cause unclean flavors. Bitterness is due to a variety of organisms, including *S. liquefaciens.* Harrison [120] found that a bitter condition in milk delivered to a cheese factory was caused by a lactose-fermenting yeast, which he named *Torula amara.* Organisms producing fishiness in milk and cream have been isolated. Fellers [121] reported that *Actinomyces* organisms may cause an objectionable bitter-moldy taste in milk after some hours. Two outbreaks of bitter-moldy milk in one dairy were traced to the presence of large numbers of the organisms; the source probably was hay and straw.

Occasionally, milk and cream have an odor suggestive of potatoes. From such cream Brannon [122] isolated an organism which reproduced the odor in freshly separated cream. Olson and Hammer [123] studied an outbreak of potato odor in milk from a certain farm and found the causative organism was *Pseudomonas graveolens* which was originally isolated from musty eggs. The organism failed to grow at 37 C (98.6 F). In aseptic milk inoculated with it and held at about 8 C (46.4 F), the count increased from 12,900 per milliliter immediately after inoculation to 5,500,000 after 96 hr when the potato odor was very evident. Pasteurization at 62.8 C (145 F) for 30 min did not decrease the intensity of the potato odor in milk in which the odor had been produced through inoculation. Olson and Hammer [123] identified as *Pseudomonas mucidolens* another organism that was isolated from cream that had developed a potato odor at a relatively low temperture; it also originally was isolated from musty eggs.

Claydon [124] reported an outbreak of medicinal flavor in pasteurized market milk that was caused by *A. aerogenes.* It occurred during late spring and considerable defective milk was returned to the plant. The organism isolated reproduced the typical defect readily in pasteurized and sterilized milk. Hand-washed bottles appeared to be the immediate source of the organism and improved sanitation in the bottle cleaning controlled the outbreak. The organism did not survive 61.7 C (143 F) for 15 min.

Many of the off flavors produced in milk and cream by microorganisms are rather temporary and are replaced by others that often are still more objectionable. With an extended holding period there are certain sequences that occur with considerable regularity; for example, a fishy flavor in cream commonly is followed by a metallic flavor. In sour cream that has been held for extended periods cheesy, rancid, and yeasty flavors are common.

The flavors produced in milk and cream by micro-organisms occasionally are very pleasant, although decidedly abnormal; fruity flavors are perhaps the best examples. In general, these pleasant flavors are quickly replaced by others that are objectionable.

The temperature of holding has a great influence on the flavors that develop. Temperatures such as 0 C (32 F) largely prevent growth of *S. lactis* and thus permit activity of certain organisms sensitive to acid that are capable of causing protein decomposition and fat hydrolysis. Small differences in the holding temperature often result in wide differences in the flavor produced, because of variations in the comparative growth rates of the organisms present.

In some instances organisms which are classified as belonging to the same species produce different flavors in milk. This indicates that a small variation in the action of an organism, which appears unimportant in its general reactions and classification, may be very significant from the standpoint of the flavor produced.

Associative action. With the flavor fermentations, as with so many other changes in milk and cream, associative action of organisms is important. Flavors that, from their manner of development, evidently are the result of bacterial action sometimes cannot be reproduced with pure cultures, whereas mixed cultures produce the condition. The difficulties encountered in attempting to develop certain flavors experimentally suggest that the balance between the causative organisms often is a delicate one. Anaerobes that cannot grow in milk in pure culture may develop in impure culture through action of other types in using the oxygen present. Study of the flavors due to organisms is greatly complicated by the necessity of sterilizing the milk and cream used for inoculation purposes, since this seriously modifies the flavor of both the uninoculated and fermented milk.

COLOR FERMENTATIONS

Action of organisms may bring about changes in the color of milk and cream. Although such fermentations have been noted occasionally in Europe, they are of little practical importance in the United States, even in milk and cream held for extended periods.

Many organisms commonly present in milk produce colored colonies, especially yellow, orange, or red, on various solid media, but development of color under these conditions is quite different from color production in a typical color fermentation. Some of the organisms forming colored colonies show no color when grown in milk; others show only a colored sediment or surface ring consisting of a mass of cells. In a typical color fermentation the color is distributed through considerable portions of the milk and is not confined to masses of growth. In general, the color changes involve the surface of milk or cream because of the oxygen requirements of the causative species. With the blue fermentation due to *Pseudomonas cyanogenes,* and possibly with other color fermentations, associative action is important.

Blue fermentation. Although various organisms have been responsible for blue coloration in milk, the one most frequently isolated appears to be *Ps. cyanogenes.* The color produced, which often is intense, is formed only in the presence of acid.

A small outbreak [125] of blue milk was reported from an Iowa city by a dealer supplying 18 families. The color was observed by the consumers three times, twice in one household and once in another. The two families were related and kitchen utensils frequently were exchanged. The color was not observed on the producing farm, except in one instance when the container used was brought from one of the homes in which the blue color had been noted. Conditions on the farm were normal and the organisms in the milk apparently came from the usual sources. In all instances, the blue color was observed in sour milk and appeared in areas at the surface. The important characteristics indicating that the abnormality was due to an organism are: (1) It developed on milk that was held; (2) it appeared in only part of the milk supplied by the dealer; and (3) the change was observed at the producing farm only in milk held in a container brought from one of the households experiencing the defect. When milk showing the blue color was studied in the laboratory, the organism found most abundant was *S. lactis* but another type, which proved to be *Ps. cyanogenes,* also was present in considerable numbers. Neither species alone gave a blue color but a mixture of the two did. The history of the outbreak suggests that the original infection of the milk with *Ps. cyanogenes* occurred in one of the households noting the defect. After the organism was established it probably was carried to the other house through the transfer of utensils.

Characteristics of *Ps. cyanogenes.* The striking characteristic of the blue fermentation of milk due to *Ps. cyanogenes* is that it does not occur except when acid is present. In pure culture in milk the organism fails to yield a blue color but a brownish tinge may develop. *Ps. cyanogenes* tends to produce an alkaline reaction in milk, and in combination with an acid-producing organism it may grow longer than it would alone, since the acid neutralizes the alkaline products formed.

The organism yields material that presumably acts as an indicator and shows a definite blue color in the presence of acid; however, addition of acid to milk in which *Ps. cyanogenes* has grown alone does not result in the color change. Importance of acid in the color production is shown by development of both acid and color in pure cultures of the organism in dextrose bouillon. A blue color can be produced in lactose bouillon by inoculating *S. lactis,* or some other acid-producing species, along with *Ps. cyanogenes.*

Blue milk due to non-bacterial causes. Blue color in milk may result from causes other than growth of bacteria. A low content of solids and the eating of certain plants by the cows are among such causes; with these, the bluish tinge is evident when the milk is drawn although it is more pronounced after the fat separates.

Yellow fermentation. Yellow color sometimes is produced in milk through bacterial action and the most common causative organism appears to be *Pseudomonas synxantha.* This species has been found in the United States, but only incidentally, and apparently has not been noted under practical conditions as a cause of color changes; it has been isolated at the Iowa Agricultural Experiment Station several times, usually from milk that had stood at room temperature until yellow color developed at the surface of the cream.

With *Ps. synxantha* [126] the yellow color is pronounced in the cream layer of milk, and the typical color change appears to be associated with the fat. The organism proteolyzes milk and the digested material may have a yellow tinge, but a conspicuous color is found only in the fat layer. Sometimes black granules also are present in the fat; undoubtedly, these consist of some decomposition product formed by the organism. *Ps. synxantha* produces a disagreeable flavor in milk and the digested material may become viscous. The yellow color of butter can be intensified by growing *Ps. synxantha* in the cream from which it is made but the color development is accompanied by a very disagreeable flavor.

Various organisms digest milk with formation of yellow color but these cannot be regarded as of significance since the digestion usually requires considerable time and involves a pronounced change in appearance and composition of the milk.

Yellow milk due to non-bacterial causes. The normal milk of certain animals, especially Guernseys, shows a yellow color which varies in intensity with the season. Such feeds as carrots and mangels sometimes cause a pronounced yellow color in milk. When there is an udder infection the milk may have a yellow tinge.

Red fermentation. Red color is produced in milk by a number of species, such as *Serratia marcescens,* growing in pure culture; although these organisms occasionally are present in milk and cream, more rapid growth of other forms usually prevents their development.

Sour milk or cream, when held for considerable periods, may show red spots on the surface which are colonies of pink yeasts; the most common of these is *Torula glutinis.* Pink yeasts[127] frequently are present in milk and cream and are not restrained by the usual acidities developed; however, they produce red spots only occasionally because of their comparatively slow growth. In some instances pink yeasts enter dairy products from the air.

Red color in milk due to action of organisms must be distinguished from red color due to presence of blood. A ruptured vessel in the udder may result in blood in the milk, and if it is present in considerable amounts the color of the milk is changed. On standing, some of the erythrocytes settle to the bottom while many are carried up with the cream, especially that making up the lower portions of the cream layer. Blood sometimes is present in milk in such small amounts that the color is not perceptibly changed, but such a condition often can be detected by centrifuging a small volume of the milk, when the erythrocytes are thrown into a distinct layer at the bottom of the centrifuge tube.

A red tinge in the glass has occasionally been the cause of a bottle of milk appearing to have a pink color; the cream especially appears pink since it provides a more definite background for the slight color in the glass.

YEASTS AND MOLDS IN MILK

Although bacteria are primarily responsible for the fermentations of milk and cream under practical conditions, yeasts and molds normally are present in the products. The numbers per milliliter vary widely, depending on the conditions of production and holding. With long holding at comparatively high temperatures the organisms are relatively numerous, owing to their ability to grow in the presence of considerable acid, and in the extreme condition masses of growth are evident on the surface of acid milk or cream.

Yeasts. The yeasts in milk and cream are of a variety of types. Except for the lactose-fermenting species and certain types that rapidly proteolyze, they are characterized by their relatively slow action on milk. Over an extended period many of them tend to sweet-curdle milk and produce an alkaline reaction.

Molds. The molds in milk and cream also are of various types. The mold most often present is *Oospora lactis* and it develops so frequently on the surface of milk or cream allowed to stand at favorable temperatures that it is regarded as the common mold of milk. With extended growth, the mold forms a heavy wrinkled membrane that is very characteristic.

Thom [128] studied the general manner of growth of *O. lactis* on dairy products and the method of reproduction. Recent studies have indicated great variations in the cultures commonly classed as *O. lactis*. Macy and Gibson [129] studied 61 cultures isolated from butter and found differences in colony size and appearance, size of conidia, growth temperatures, and heat resistance. All of the cultures liquefied gelatin and hydrolyzed cottonseed oil and butter fat, but none of them produced acid in litmus milk. The flavors produced in skim milk, whole milk, cream, and lactic acid culture were not pronounced, except with 6 cultures which gave fruity or cheesy flavors.

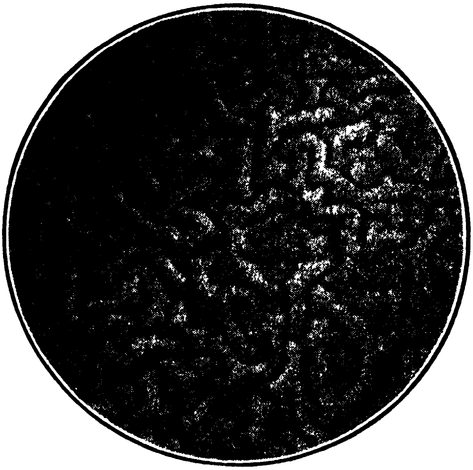

Fig. 17. Wrinkled surface growth of *Oospora lactis* on cream. Natural size. Courtesy R. V. Hussong.

In the trials of Friedemann,[130] *O. lactis* in a medium prepared from 0.3 per cent meat extract or an infusion of beef muscle to which were added 1 per cent peptone, 1 to 1.8 per cent disodium phosphate crystals, and 0.9 per cent dextrose yielded about 70 per cent lactic acid on the basis of the sugar consumed. Approximately one-fifth of the C_3 intermediates from the sugar were further metabolized to formic acid, acetic acid, and ethyl alcohol.

When growing on the surface of sour milk or cream, *O. lactis* tends to lower the acidity by using some of the lactic acid in its metabolism and by neutralizing some of it with certain growth products. The reduction in acidity eventually may permit development of other organisms, especially bacteria, that were restrained by the higher acidity.

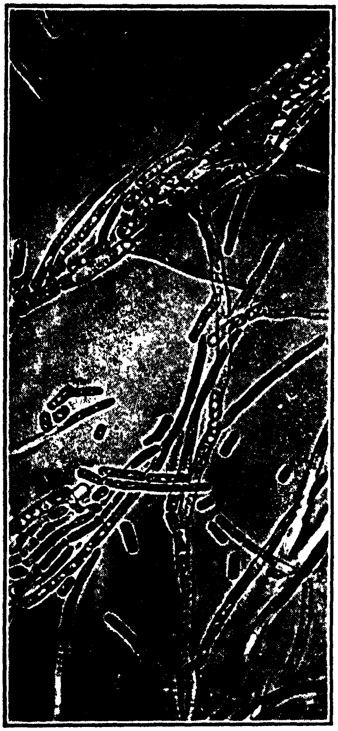

Fig. 18. *Oospora lactis.* Courtesy E. H. Parfitt.

Because of the frequency with which *O. lactis* is present in milk, its source is of importance. Garrison[131] reported that cow manure is the major source of *O. lactis* on dairy farms. It is distributed widely by manure to various places about the barn and surroundings. Dust raised in the barn by grooming the cows usually contained the organism in significant numbers. Dust from hay and bedding contained relatively small numbers. On farms producing cream, the organism was isolated from 80.6 per cent of 36 separators, 76.7 per cent of 60 milk pails, and 65.2 per cent of 23 strainers. On farms marketing milk, the milk tubes and teat cups of 5.1 per cent of 39 milking machines yielded the organism. It was detected in various feeds, soils, and in water.

O. lactis is easily destroyed by heat. Tanner and Hofer[132] found with 224 cultures, from a wide area, that none survived 62.8 C (145 F) for 30 min. Of 144 cultures exposed in cream to 57.2 C (135 F) for 30 min, 8 survived; 51.7 C (125 F) for 30 min did not kill any of the cultures.

Molds other than *O. lactis* that are present in milk and cream come from a variety of sources. In many instances the spores are carried to the products through the air.

NUMBERS OF ORGANISMS REQUIRED TO PRODUCE DEFINITE CHANGES IN MILK

Milk may contain large numbers of bacteria per milliliter without showing abnormalities and this suggests that, in general, large numbers of organisms are required to cause definite defects. Hammer and Hix[133] inoculated aseptic or sterile milk with various organisms and made plate counts as defects developed. When an increased acidity recognizable by the sense of taste was produced by *S. lactis* in aseptic milk, the counts varied from 30 to 90 million per milliliter (7 trials); when the milk was regarded as sour the counts ranged from 53 to 166.5 million (4 trials). A slightly ropy condition produced by *Alc. viscosus* in sterile milk in thin layers required from 15.5 to 44 million organisms per milliliter (19 trials); with a very ropy condition the counts ranged from 450 to 786 million (4 trials). Comparable conditions produced by *A. aerogenes* involved counts of 11 to 49 million (7 trials), and of 290 to 475 million (37 trials), respectively. Bitter flavor in aseptic milk was produced by an organism characterized by the development of this defect with counts from 10 to 66 million per milliliter (10 trials). When a sweet curdler produced slight coagulation in sterile milk the counts ranged from 1.25 to 4.9 million per milliliter (21 trials), and when there was firm coagulation the counts varied from 15.9 to 56 million (26 trials). A faint odor was produced in sterile milk by an unidentified organism with counts of 14 to 57 million per milliliter (20 trials). In the tests the sweet curdling organism produced a change in milk with the smallest number of organisms.

At the Iowa Agricultural Experiment Station the numbers of yeasts in cream showing various conditions were determined. On cream with a yeasty odor the plate counts were 1.6 and 2.2 million per milliliter, on cream with a decidedly yeasty odor they were 10.6 and 12.8 million, and on foaming cream they were 6.3 and 11.2 million.

Other data also indicate that relatively large numbers of organisms ordinarily are required to produce definite changes in milk. Curran[134] found with a sweet curdler that coagulation of milk occurred when a population of 10 to 40 million per milliliter was attained. Olson and Hammer[123] noted that aseptic milk inoculated with *Ps. graveolens* had a pronounced potato odor with a count of 5.5 million per milliliter. Gould and Jensen[16] reported that microscopic clump counts on milk samples showing slight acid-associated flavors ranged from 1.5 to 10 million.

In considering the number of organisms required to produce a change in milk, the uneven distribution of organisms under practical conditions must be recognized. When ropiness is present only in the cream layer of a bottle of milk, the number of bacteria per milliliter in the cream is high, whereas in the milk beneath there may be relatively few organisms. Because of the comparatively small volume of the cream, the count on the milk after thorough distribution of the cream may be relatively low.

CHARACTERISTICS OF MILK ABNORMALITIES DUE TO ORGANISMS

Abnormal conditions in milk due to growth of organisms have certain characteristics that ordinarily render them distinguishable from abnormalities due to other causes, and frequently consideration of the general nature of an unusual condition makes it possible to determine whether organisms are responsible. The more important of these characteristics are as follows:

1. The abnormal condition is not present when the milk is drawn. A defect in milk, such as ropiness or an abnormal color, that is due to activity of organisms requires an opportunity for growth; but comparable abnormalities due to the condition of the animal are present when the milk is first drawn. A flavor caused by action of organisms is not present at the time of milking; one due to feed of the animal is present. When this character is used in connection with flavors in milk, the possibility of absorption from the surroundings must be considered.

2. The abnormal condition usually increases on standing. In general, as a result of increased growth of the causative organism, the abnormal condition becomes more conspicuous. For example, ropiness becomes more pronounced with longer holding, if the temperature remains favorable for the organisms. Eventually, there may be a disappearance of any abnormal condition, whether due to organisms or other causes, because of extensive growth of various species.

3. In many instances the abnormal condition can be inoculated from one lot of milk to another. Organisms causing an abnormality in milk often produce the same condition when a bit of the milk is transferred to a fresh lot, preferably sterile milk. However, a number of factors, such as growth of organisms other than the causative species and use of unsatisfactory temperatures, can be responsible for failures in such trials. When reproduction of an abnormal condition is attempted, it is advisable to work with cultures, either pure or in known mixtures, rather than with the abnormal material direct.

Important exceptions. Rancid and bitter flavors in the milk of certain animals advanced in lactation and oxidized and tallowy flavors caused by various factors show certain characters suggesting that they are due to organisms; for example, they increase on standing and commonly are not present when the milk is first drawn. However, these flavors are so characteristic that they are readily recognized.

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Chapter 4

Contamination of Milk and Its Control

During production and handling of milk, micro-organisms ordinarily are added to it from a variety of sources. The numbers contained when milk leaves the stable vary from a few thousand per milliliter, or even less than 1,000, under controlled methods of production, to 500,000, or more, when unsatisfactory conditions prevail. In the plants handling milk and in the homes in which it is consumed, addition of organisms continues, although with proper care this contamination is slight. Temperature and period of holding largely determine the extent of the growth that occurs, but the contamination provides the initial flora and thus assumes an importance beyond the actual number of organisms added.

Because of differences in the general character and importance of the organisms involved, the contamination of milk can be logically divided into (1) contamination from interior of the udder, and (2) contamination from external sources.

CONTAMINATION FROM INTERIOR OF THE UDDER

At one time it was commonly believed that milk, especially that drawn toward the close of a milking, could be obtained in a sterile condition. The idea was not the result of careful investigation; it was based largely on trials in which a few milliliters of apparently sterile milk were collected and on the fact that milk obtained aseptically often shows but little tendency to undergo rapid or conspicuous changes. Both Moore[1] and Ward[2,3] secured results which make the idea untenable; they examined udders of cows killed after being milked dry and found that organisms could be obtained regularly from the glandular tissue. The types recovered seemed to be identical with certain of those found in aseptically drawn milk. Ward examined 19 udders and reported that the lactiferous ducts harbored bacteria throughout their whole extent. He concluded: "Milk, when secreted by the glands of the healthy udder, is sterile. It may, however, immediately become contaminated by the bacteria which are normally present in the smaller milk ducts of the udder."

It is now generally agreed that milk contains bacteria at the time it is drawn as a result of contamination in the milk ducts and cistern. Growth of organisms in these passages during the periods between milkings provides the supply that makes possible continued contamination of the milk.

Numbers of bacteria in milk drawn aseptically. The organisms in milk drawn aseptically are difficult to estimate accurately; the tendency to form clumps suggests unusually large errors with the plate method, and the numbers ordinarily are too small for satisfactory microscopic counts. The plate method has been used most often in the examinations reported.

Harding and Wilson [4] presented data on milk obtained under controlled conditions from 78 cows, the samples commonly being secured at the close of the milking: 316 samples from the New York (Geneva) Agricultural Experiment Station herd during 1900 to 1902 had an average bacterial count of 518 per milliliter; 730 samples from the same herd during 1910 and 1911 had a count of 420; 184 samples from a farm herd had a count of 320. The average count for the 1,230 samples was 428 per milliliter. Only 8 per cent of the samples contained more than 1,000 bacteria per milliliter.

Copeland and Olson [5] determined the numbers of bacteria in milk from each quarter of 40 cows, the samples being taken after approximately one-third of the milk had been drawn from the quarters. Omitting unusually high counts on milk of one animal, the average number was 1,541 per milliliter. Breed,[6] using a dilution of 1 to 10 in pouring the plates, found an average bacterial content of 964 per milliliter for 45 samples of strippings drawn directly into sterile tubes from individual quarters; in 8 instances the counts were between 1,000 and 15,000.

Dorner,[7] working at Geneva, N. Y., examined 993 samples of milk from 132 cows belonging to 6 herds. The calculated average bacterial counts for the milk from the various herds showed wide variations. With the Burri smear culture technic the lowest herd count was 3,965 per milliliter and the highest was 9,635; the average for all the herds was 7,475. With the plate method the herd counts varied from 530 per milliliter to 4,390 and averaged 2,775. In subsequent work at Liebefeld, Switzerland, Dorner examined 944 samples of milk from 241 cows in 22 herds with the Burri technic. The average counts for the different herds varied from 861 per milliliter to 10,564; the average for all the herds was 3,099.

The average bacterial count on 284 samples of aseptically drawn milk examined by Wayne and Macy [8] was 658 per milliliter. In Utah, Jones and Stevens [9] selected 10 cows that consistently gave negative tests for tuberculosis, brucellosis, and mastitis and never had shown any symptoms of infectious mastitis. Over a 4-mo period, milk from each quarter of the udders of the 10 cows gave an average of 239 bacteria per milliliter, with a minimum of 49 and a maximum of 511.

Thornton and Strynadka [10] examined aseptically drawn milk with the microscopic method, using 1,000 fields for a determination. Each sample represented about equal amounts of milk from each milking quarter taken after about half the milk had been drawn; 88 samples of macroscopically normal milk were studied. With the 31 samples having leucocyte counts not over 500,000 per milliliter, the microscopic counts averaged 34,980 per milliliter, with a minimum of 3,000 and a maximum of 231,600; 10 samples gave counts under 10,000 and 2 gave counts over 100,000. On 17 of these samples the plate counts averaged 918 per milliliter, with a maximum of 4,600. With the 57 samples having leucocyte counts over 500,000 per milliliter, the microscopic counts averaged 204,514 per milliliter, with a minimum of 3,600 and a maximum of 2,562,000; 6 of the samples had counts under 10,000 and 4 had counts over 1,000,000. Thirty-two of the 57 samples had an average plate count of 36,625 per milliliter, with a minimum of 150 and a maximum of 511,000. Rods were not identified in the samples. With the low and the high leucocyte samples, respectively, single cocci were found in 35.5 and 15.8 per cent; groups no larger than diplococci in 38.7 and 24.6 per cent; streptococci in 16.6 and 42.1 per cent; and non-chain groups in 22.6 and 26.3 per cent.

There commonly is considerable variation in the counts on milk from different animals and this suggests that milk having counts decidedly lower than the averages might be obtained. Such milk has been reported from various sources. Samples submitted to milk-scoring contests have shown many very low counts, sometimes under 100 per milliliter. Atwood and Giddings [11] found an average of 35 bacteria per milliliter and extremes of 6 and 120 for 6 samples of approximately 2 qt each that came from one cow; only middle milk from the rear quarters was included.

From the data available, it is evident that plate counts on milk drawn aseptically from normal udders commonly are low. Presumably, with udder infections, the counts may be considerably higher. General observations indicate that in some instances the ratio between plate and microscopic counts on aseptically drawn milk is rather wide.

Excessive counts on milk drawn aseptically. Some animals with apparently normal udders give milk that commonly contains unusually large numbers of bacteria. Two such cows were studied by Hastings and Hoffmann;[12] the results, together with data obtained on a third animal giving milk containing normal numbers of bacteria, are as follows:

Excessive Bacterial Counts on Milk Drawn Aseptically

Cow	Number of Samples	Bacteria per Milliliter		
		Average	*Minimum*	*Maximum*
Brownie	61, covering 15 mo and parts of 2 lactation periods	30,700	1,700	305,000
Dorine	31, excluding 2 very high counts	38,800	2,500	154,000
Dorine	33	191,000 *	2,500	3,500,000
Merney	27	800	50	4,250

* See ref. 4.

Copeland and Olson[5] made numerous counts, during one lactation period, on the milk of a cow with an apparently normal udder and found that 40 exceeded 50,000 per milliliter and 17 exceeded 100,000; the highest count obtained was 347,000.

Cows giving milk containing excessive numbers of bacteria undoubtedly are of significance in the production of milk that must comply with rigid bacterial standards. In some instances failure of certified milk to meet the bacteriological requirements has been due to a few cows giving exceptionally high-count milk, and the exclusion of this milk has quickly brought the numbers of organisms within the limits set. Such cows appear to have normal udders and can be detected only by running counts on milk from individual animals in the herd.

Distribution of organisms throughout the milking. There is considerable variation in the numbers of organisms per milliliter in different portions of the milking, the fore milk quite regularly containing the largest number. In a series of comparisons von Freudenreich[13] found the fore milk averaged 6,505 per milliliter, the middle milk 1,341, and the strippings 769; the fore milk did not always show the highest count in these comparisons. The following data, taken from results obtained by Stocking,[14] illustrate how the numbers of organisms decrease as the milking proceeds:

BACTERIAL CONTENT OF VARIOUS PORTIONS OF A MILKING

Number of trial	Bacteria per Milliliter				
	Streams 1 and 2	*Streams 5 and 6*	*Streams 9 and 10*	*Streams 13 and 14*	*Strippings*
1	1,940	550	250	275	216
2	25,200	5,391	285	218	101
3	5,491	2,096	430	820	144
4	7,941	1,350	125	216	156

Harding and Wilson[4] examined the milk from 5 cows on 6 successive days, the milk from each quarter being studied separately, and found the general averages to be as follows: fore milk 458 per milliliter, middle milk 187, and last milk 274.

Copeland and Olson[5] determined the numbers of bacteria in the fore milk, middle milk, and strippings from each quarter of 8 cows. Omitting the counts from one infected quarter, the fore milk averaged 5,989 per milliliter, the middle milk 557, and the strippings 415. The bacterial content of fore milk was studied in detail by Faber.[15] The first stream from each quarter of the udder was drawn into a sterile bottle and the procedure then repeated with other streams. The average bacterial counts obtained in 100 series of analyses were first stream 1,275 per milliliter, second stream 927, third stream 705, fourth stream 647, fifth stream 551, and eleventh stream 449. The first 5 streams had an average count of 821 per milliliter; the middle milk had an average count of 449.

The comparatively high bacterial content of fore milk is due to this portion of the milk washing out most of the easily removable organisms present in the milk passages. Contamination from these sources persists during the remainder of the milking through the gradual dislodging of organisms but is less per milliliter of milk. In the instances in which the strippings have a higher bacterial content than the middle milk, manipulation of the udder incident to the stripping may have caused an increased dislodging of organisms.

Effect of elimination of fore milk on bacterial count. Since the fore milk contains more bacteria per milliliter than the remainder of the milking, elimination of the fore milk has been suggested as a means of reducing contamination from the interior of the udder and is practiced in some dairies interested in keeping the bacterial content of the milk low. The usual procedure is to have someone go ahead of the milkers and draw the first few streams from each teat into a container, this milk then being discarded or used for feeding purposes. The fore milk should not be drawn on the floor since its fermentation there may cause undesirable odors in the stable.

In eight comparisons using two lots of 5 cows each, Stocking[14] obtained average counts of 522 per milliliter when the fore milk was not rejected and 499 when it was rejected, a decrease of about 4 per cent. In tests at the Iowa Agricultural Experiment Station discarding the fore milk reduced the bacterial count about 4 per cent. The following results were obtained by Lochhead:[16]

EFFECT OF DISCARDING FORE MILK ON BACTERIAL COUNT OF MILK

	Bacteria per Milliliter	
	Fore Milk Not Discarded	*Fore Milk Discarded*
Sanitary conditions of production	3,770	3,200
Insanitary conditions of production	397,600	393,800

The small reduction in bacterial count due to discarding the fore milk is in agreement with the small volume of this portion of the milking. Elimination of the fore milk may be of value when the general conditions of production are excellent and very low bacterial counts are desired; it is of no significance with unsatisfactory conditions of production. Another point to be considered is the flavor of the fore milk; frequently, this is unsatisfactory and discarding the fore milk may be an advantage if milk with an exceptionally fine flavor is desired. Rejection of the fore milk involves little fat loss because this portion of the milking is comparatively low in fat.

Numbers of bacteria in milk from different quarters. Data obtained by various investigators show that, commonly, there are wide variations in bacterial counts on milk from the different quarters. In the extreme cases less than 10 bacteria per milliliter are present in milk from some quarters, whereas several thousand are contained in that from other quarters. Since the bacterial count on the entire milking is a composite of the bacterial counts on the milk from the different quarters, unusually low or high counts on milk from one quarter are counterbalanced by milk from the other quarters. Most investigators have noted no regularity in the quarters supplying milk showing either relatively low or high counts. Differences have been reported in the types of organisms from the different quarters of an udder.

Influence of period of lactation on numbers of bacteria. Atwood and Giddings [11] made a number of examinations of colostrum from a heifer and found the bacterial counts varied from 35 to 3,350 per milliliter. Harding and Wilson [4] reported data in which the period of lactation had no marked influence on the bacterial count on the milk, although the colostrum had a slightly higher count than milk obtained later. Copeland and Olson [5] determined numbers of organisms in colostrum from each quarter of 6 cows, the samples being collected within 6 hr of calving and in 3 cases before the calf had suckled. Most of the counts were rather high but a number were under 1,000 per milliliter; the maximum was 650,000. There were the usual variations in milk from different quarters. The investigators studied 4 cows for 14 mo, making counts every 5 days, and found that the average monthly bacterial counts tended to remain fairly constant; the results also showed that variations in bacterial counts on the milk from different quarters may be permanent. Wayne and Macy [8] examined milk from 6 cows and noted that in most cases the colostrum had a lower count than the milk drawn later; with 1 cow the colostrum had an unusually high count and the milk drawn later had a normal count.

Influence of various factors on numbers of bacteria. Age of the cow, character of the feed, and similar factors apparently have no definite influence on the bacterial count on aseptically drawn milk.

In studies on the effect of various methods of drying up cows on the bacterial content of milk, Wayne and Macy [8] found that intermittent and incomplete milking usually resulted in somewhat higher bacterial counts during such periods, and that immediately following periods of suspended milking the bacterial counts were slightly higher. None of the milking procedures used in drying up the animals appeared to have any marked effect on the bacterial content of the milk in subsequent lactation periods.

Influence of various treatments on bacteria in the udder. Atwood and Giddings [11] attempted to reduce the contamination of milk drawn under controlled conditions. After the udder had been thoroughly stripped, the end of the teat was dried with a sterile towel and then moistened with 25 per cent lysol. This was wiped off, carbolated vaseline rubbed on, and the vaseline protected with adhesive tape. Counts obtained on the milk after such treatment did not differ significantly from those obtained on milk produced normally, which indicates that contamination through the teat canal has little influence on the number of organisms coming from the interior of the udder. Injection into the udder of materials intended to destroy or inhibit bacteria caused inflammations.

Hoffmann [12] injected udders with physiological salt solution, 2 per cent boric acid solution, or distilled water and found that such inert materials produced rapid and marked leucocytosis. In some instances an increased leucocyte count was accompanied by a decreased bacterial count, which may have been due to phagocytic action since microscopic preparations showed many leucocytes containing from 5 to 25 bacteria, while leucocytes from uninjected quarters showed no such activity.

Wallis *et al.*[17] reported that sealing the teats with collodion between milkings seemed to have no noticeable effect in reducing bacterial counts on milk or preventing relatively wide fluctuations in the counts.

Types of organisms in milk drawn aseptically. The organisms present in milk drawn aseptically belong to certain more or less definite types. To multiply in the udder, an organism must have the ability to grow in contact with the tissues there, and this is a character lacking in many common species. Some of the species that develop rapidly in milk after it is drawn, die quickly when introduced into the udder. Ward [2] injected *Serratia marcescens* through the teat canal and, although an infection was set up, only a few colonies developed on plates poured after 24 hr; none developed on plates poured on the sixth day. Russell and Hastings [18] reported results on the injection into the udder of four species, the common lactic acid organism, an acid-producing liquefying form, *Ser. marcescens,* and a yellow liquefying coccus frequently present in fore milk; in all cases the numbers of the organisms recovered on plates became smaller as the period of exposure in the udder increased. In most instances the injection of an organism resulted in the milk becoming more or less abnormal and sometimes the udder was inflamed. Copeland and Olson [5] injected *A. aerogenes* into one quarter and, although this became swollen and enlarged, the organism survived only a few days.

Micrococci are particularly prominent among the organisms isolated from aseptically drawn milk and also are conspicuous among the organisms obtained from udder tissues. Evans [19] studied 192 samples of milk from 161 cows, representing five different dairies, and found micrococci in 113 (58.9%); the highest number was 80,000 per milliliter. Many of the micrococci belonged to one group, which agreed in its characteristics with the pyogenic staphylococci, but most of the cultures were non-virulent; however, two cultures possessed such high virulence that inoculated rabbits died in 16 hr. Three types of micrococci gave reactions which differed from the pyogenic staphylococci; one was identified as *M. luteus,* one was characterized by a rapid and complete peptonization of milk and was named *M. caseolyticus,* and the third was not named.

Breed[6] identified 171 of 176 cultures of micrococci isolated from strippings drawn under controlled conditions. The numbers of isolations of the various species were *M. aureus,* 33; *M. aurantiacus,* 24; *M. freudenreichii,* 23; *M. albus,* 21; *M. candidus,* 20; *M. epidermidis,* 13; *M. citreus,* 10; *M. varians,* 10; *M. flavus,* 8; *M. conglomeratus,* 4; *M. luteus,* 3; *M. casei,* 2. The remaining 5 cultures gave a red or pink growth.

The udder micrococci often produce colored colonies on agar, especially yellow or orange; chromogenesis commonly is more conspicuous on plates incubated at 21 C (69.8 F) than at 37 C (98.6 F). In general, the organisms produce changes in milk comparatively slowly and thus are of little importance in the deterioration of milk under practical conditions.

Streptococci also are present in milk from normal udders. Sherman and Hastings[20] found them in 0.01 ml of the mixed milk from 10 of 12 herds and in 38.6 per cent of the individual samples from 88 cows in 4 herds. Evans[19] obtained long-chained streptococci which were not *S. lactis* from 29 (15.1%) of 192 samples studied, the highest number being 264,000 per ml.

By means of special media, especially agar to which blood serum has been added, rod-shaped bacteria often can be isolated from milk drawn aseptically. Evans[19] recovered them from 45 (23.4%) of 192 samples of milk examined, the maximum number being 50,000 per milliliter. In another series of 23 individual samples, this investigator[21] found that 17 (73.9%) contained the rod forms, the maximum number being 112,000 per milliliter; if the 23 samples of milk had been mixed, there would have been 7,000 of the organisms per milliliter. The organisms, which grew rapidly in milk, were of different types and one is of special interest, since with high initial contamination of cream, it hydrolyzed butter fat and produced a disagreeable flavor and odor in 24 hr.

Influence of the medium used on the types of organisms isolated from aseptically drawn milk is evident from the work of Dorner.[7] With 993 samples collected at Geneva, N. Y., and plated on nutrient agar, micrococci made up 47.0 per cent of the organisms, streptococci

42.3 per cent, and rod forms 10.4 per cent. When the same samples were examined with the Burri technic, using a special medium, rod forms made up 49.9 per cent of the organisms, streptococci 32.2 per cent, and micrococci 18.4 per cent. Later, Dorner examined 944 samples obtained at Liebefeld, Switzerland, with the Burri technic and found micrococci most abundant, streptococci next in importance, and rod forms fewest in number. The difference in the results at Geneva and Liebefeld suggests there may be definite variations in the organisms prominent in aseptically drawn milk in different areas.

Knowles[22] plated samples of fore milk from 4 healthy cows on litmus lactose agar and incubated the plates at 30 C (86 F) for 40 hr; counts per milliliter were 40; 65; 3,220; and 145. The number of liquefiers per milliliter on litmus lactose gelatin plates incubated at 22 C (71.6 F) for 40 hr were 0; 7; 1,200; and 6. They included a *Staphylococcus, M. caseolyticus, M. freudenreichii,* and *S. liquefaciens.* The author concluded the fore milk may contribute to cheese acidoproteolytic organisms which aid in the ripening process.

Occasionally, organisms other than the usual udder types are present in milk as a result of contamination in the udder. In some cases they are related to abnormal udder conditions; in other instances they are organisms that have penetrated the teat canal or been brought to the udder through the blood stream and probably will soon disappear from the udder because of their inability to tolerate the conditions there. Rarely, organisms that are present in milk in considerable numbers as it leaves the udder may be involved in rapid deterioration of the product.

Pathogens in milk drawn aseptically. As a result of an infection in the producing animal, milk sometimes contains pathogens at the time it leaves the udder. The organisms causing tuberculosis, brucellosis, and certain udder infections are the most important of these in cows' milk; the organism causing brucellosis is the most serious in goats' milk. Presence of these organisms in milk and their general importance are discussed in Chapter 8.

CONTAMINATION FROM EXTERNAL SOURCES

Contamination of milk from external sources can be divided conveniently into (1) that taking place on the producing farms, and (2) that occurring subsequent to production and involving especially contamination in the plants handling milk.

CONTAMINATION ON PRODUCING FARMS

On the producing farms the sources from which micro-organisms are added to milk include (1) stable air, (2) utensils, (3) coat of the animal, (4) milker, and (5) miscellaneous sources.

1. STABLE AIR

Stable air frequently contains dust in considerable quantities, and this fact has led to the assumption that air is an important factor in the contamination of milk. Investigations have shown, however, that, although some organisms enter milk from this source, the numbers ordinarily are not large; it is probable that the desiccation and light to which the organisms carried by the dust are subjected destroy many of them so that material falling into milk from the air adds comparatively few living organisms.

Methods of measuring contamination from the air. The extent of contamination of milk from the air has been investigated in various ways. One of the simplest methods is to expose agar plates of a known area for a given period in the stable to be studied and then to calculate, from the colonies developing during incubation, the number of organisms that would fall into a pail with a certain opening during the milking period. The method is not at all satisfactory since the organisms present on a dust particle may be so close together that several develop into a single colony, and it also is probable that some organisms are held away from the surface of the agar and do not develop at all. Sterile milk or water can be exposed in a pail and the organisms falling into it from the air determined by making counts. Variations in air conditions can be shown by determination of the organisms in air with an aeroscope, air centrifuge, or other device but such data give no direct information on the organisms that would be added to milk. Ruehle and Kulp [23] devised an "artificial udder" from which sterile water can be "milked"; the bacterial content of the water then is determined.

The effect, on the bacterial content of milk, of increasing the dust in the air by some such process as feeding can be investigated by milking half of a group of cows when the air is comparatively free of dust and the other half after carrying out the operation being studied; samples for the counts are taken as soon as the milk is drawn. The two lots of cows are alternated in different comparisons to compensate, as far as possible, for variations in contamination from the interiors of the udders.

Dusty stable air. Lochhead[16] obtained the following results in studying the organisms in dusty stable air:

ORGANISMS IN DUSTY STABLE AIR

	Bacteria Falling per Minute into Pail 11 In. in Diameter	Calculated Contamination per Milliliter of Milk on Basis of 10 Lb Drawn in 8 Min
No sweeping or handling of hay 1 hr before milking	10,800	22
Feeding hay 10 min before milking	23,300	45
Cleaning and sweeping 10 min before milking	31,600	62

Influence of feeding just before milking. Stocking[14] studied the influence of feeding hay and grain just before milking on the bacterial content of milk, using 10 cows in six comparisons and determining the organisms by the plate method. When the cows were milked before feeding, the numbers of total, acid-producing, and liquefying bacteria per milliliter were 2,096; 790; and 108, respectively. When the cows were milked after feeding, corresponding counts were 3,506; 1,320; and 196. The influence of feeding dry corn stover also was studied by Stocking, using 2 cows in five comparisons. With milking previous to feeding, numbers of total, acid-producing, and liquefying bacteria per milliliter were 1,233; 297; and 1, respectively. With milking subsequent to feeding, corresponding counts were 3,656; 692; and 28.

Influence of brushing animals just before milking. The influence of the dust raised by brushing the cows just before milking on the bacterial content of milk was studied by Stocking,[14] using 10 cows in 14 comparisons. When the cows were not brushed, numbers of the total, acid-producing, and liquefying bacteria per milliliter were 1,207; 213; and 59, respectively. When the cows were brushed just before milking, corresponding counts were 2,286; 381; and 117.

Bacterial content of stable air. Ruehle and Kulp[23] investigated the bacterial content of stable air and its effect on the bacterial content of milk, using aeroscopes and an "artificial udder" from which sterile water could be "milked." When the cows were in the stable the germ content of the air usually was between 50 and 100 per liter, with a maximum of 825. Sterile water "milked" under these conditions had an average bacterial content of 12 per milliliter, the highest count being 73. When a heavy dust was raised in the stable loft by sweeping up material from the floor, the bacterial content of the air was from

1,000 to 2,000 per liter and sterile water "milked" under these conditions had an average bacterial content of 47.6 per milliliter. By raising dust continuously during the test the bacterial content of the air increased to between 2,500 and 10,000 per liter and the average count of the water "milked" was 604 per milliliter. Fifty-eight analyses of the air in three commercial laboratories gave results in agreement with those obtained in the stable investigated, except in four instances in which the results were like those obtained in the dusty loft. On exposing sterile liquid to the air of a stable for 5 min, the counts indicated an average of 55 organisms for each square centimeter of surface when chopped hay was being fed, 71 when dry grain was being fed, and 114 when milking was in progress. Under these conditions, if 5 liters of milk had been exposed in an open 12-in. pail for 1 hr the organisms added to the milk per milliliter would have been 99; 124; and 199, respectively. On raising a heavy dust in the stable loft, the average number of organisms per square centimeter, after a 5-min exposure, was 678 when the dust was raised but once and 28,377 when the dust was stirred up continuously. The bacterial content of a pail of milk exposed to such heavily contaminated air would be increased from 1 to several thousand per milliliter in a few minutes.

General effect of contamination from air. Although under extremely dusty conditions, significant numbers of organisms are added to milk from the stable air, the numbers normally added are not large, even when operations that tend to throw dust into the air are carried out shortly before milking. If rigid bacterial standards are being met, contamination from the air definitely should be considered.

Reducing contamination from stable air. Reduction of the contamination from stable air involves primarily a logical selection of the time for such operations as brushing, feeding, and bedding the animals so that the air will be reasonably free of dust during the milking. In general, feeding of concentrates just before milking is much less objectionable than feeding of roughage. It is easier to keep the stable air in a satisfactory condition when the floors, walls, and ceiling are clean than when they are covered with dust and dirt. With an excessive amount of dust in the air, considerable material settles into the milk and has an influence on the amount of visible dirt present, as well as the number of micro-organisms.

Types of organisms from air. Because of the severe conditions existing in the air, most of the organisms coming from this source must be comparatively resistant. They include spore-forming bacteria, both aerobic and anaerobic, certain cocci, especially micrococci, and various yeasts and molds. Organisms that have very little resistance sometimes are encountered; presumably they have been in the air only a short time.

2. UTENSILS

Unless the utensils with which milk comes in contact are steamed, scalded, or treated in some other way which largely destroys the organisms in them, they often are serious sources of contamination. On a farm, if little attention is given the utensils, they may be the most important source of bacteria in the milk.

Methods of measuring contamination from utensils. A common method of measuring contamination from a utensil is to add a known volume of sterile non-toxic tap water, buffered distilled water, or comparable material, suspend the organisms in it as completely as possible by shaking or with a sterile brush, determine the bacteria per milliliter of the rinse material, and calculate the number in the utensil.[24] If preferred, the result can be calculated as the number of organisms that would be added per milliliter if the container were filled with milk. The general procedure has been modified in various ways, depending on the utensil and other factors. Results are low because of the difficulty in dislodging organisms and breaking up clumps. Organisms from utensils in which growth has occurred often are in clumps.

A contact plate procedure has been developed for approximating the numbers of organisms on flat surfaces.[24] A layer of agar supported by a tin can cover is pressed lightly against the surface to be examined and then held in a sterile petri dish. After incubation the colonies are counted.

Various swab procedures have been devised in which a swab is rubbed over an area to pick up organisms.[24] The swab then is treated in various ways to disperse the organisms in a dilution blank and the blank plated. Swab test methods are proving useful in examination of utensils for various foods and equipment in food processing plants.

Jamieson and Chen [25] swabbed the surfaces of equipment with sterile cotton swabs, which were moistened with sterile water for use on dry surfaces, and then smeared each swab on a suitable medium in slanted oval test tubes or in flat bottles with caps. Reliability of the test was substantiated by statistical studies. The test can be carried out readily in a plant with transported equipment.

Olson and Hammer [26] suggested an agar disc method for studying contamination from utensils and dairy plant equipment. The medium is made with 2.5 per cent air-dried agar so the disc will be strong enough to permit handling; the other ingredients can be those used for standard beef infusion, or any type of agar medium. After liquefying and cooling the medium to about 43 C (109.4 F), a small amount is poured on the area to be studied and allowed to solidify. The disc thus formed is picked up with a sterile spatula and tipped into a sterile petri dish so that the portion in contact with the surface being examined is toward the top of the dish. After incubation under the conditions desired, the colonies developing on a measured area (usually 20 to 30 sq cm) are counted and the results expressed as the number per square centimeter. With heavily seeded discs, approximate counts or estimates are necessary. The method is useful primarily for comparative purposes. It gives a general picture of the surface examined from the standpoint of numbers, distribution, and colony types of the organisms present; such a picture often is more impressive than bacterial counts on water used to rinse the surface. Discs cannot be prepared in inaccessible places, where proper cleaning is especially difficult, but by tipping a utensil most surfaces can be put in such a position that discs can be obtained.

Numbers of organisms from utensils. The early studies clearly indicated the importance of utensils as a source of organisms. Prucha *et al.*[27] found that 170 freshly washed and unsteamed cans contained bacteria sufficient to have added from 197 to 2,557,000 organisms per milliliter, with an average of 128,592, if the cans had been filled with milk. Investigation of 91 cans that were washed, rinsed, and steamed at the plant and then covered with their lids and returned to the farms showed that, as they were about to be used, they would have added 23,523 bacteria per milliliter to the milk; treatment on the farms was not uniform, either as to care of the cans or period of holding. The collective influence of the utensils was studied on 81 cans of milk drawn and handled with sterile utensils in comparison with 117 cans for which the utensils were not sterilized; average bacterial content of the former was 6,807 per milliliter and of the latter 285,600.

With a shipment of 4,000 gal of milk, North[28] obtained the following bacterial counts: milk from 60 farms at shipping station 5,000,000 per milliliter; milk after pasteurization 6,700; pasteurized milk 3 min after adding to unsterile 10-gal cans 560,000; milk in New York City the following day 12,000,000.

In the examination of 236 cans under all conditions of washing and steaming, Smith[29] found that, had the cans been filled with milk, contamination would have varied from 0 to 7,920,000 organisms per milliliter. Later, Smith[30] reported additional data. Cans were examined at the railway station as they were being sent to shippers; 108 wet cans would have added 547,994 bacteria per milliliter of milk, with extremes of 52 and 4,332,000, whereas 38 dry cans would have added 1,870 bacteria, with variations from 2 to 19,632. Sixty cans from four plants using washing machines and air blasts for washing and drying the cans would have added 14 bacteria per milliliter of milk, with extremes of 0 and 331. In one plant the effect of improved washing equipment on the condition of the cans was clearly evident; with the old method 9 cans would have added 88,500 bacteria per milliliter of milk, with variations from 23,700 to 178,000, whereas after a can-washing machine with an attached air blast was installed 12 cans would have added 122 bacteria, with variations from 52 to 634.

Shutt[31] found that samples of milk collected on a farm immediately after drawing into farm-sterilized pails had an average count of 700 per milliliter but that when the same milk was poured into a can received from a dairy plant and held about 12 hr in a cooler the count was 5,480,000.

Growth of organisms in utensils. The enormous numbers of organisms in certain utensils can be explained only on the basis of growth of organisms in them. Extent of the growth is determined by the conditions and the species of organisms involved. Water containing traces of milk solids provides a suitable medium for many species, and the temperatures at which utensils often are held, especially during warm months, are satisfactory for rapid development. When the washing is carelessly done so that milk solids remain on the utensils in considerable amounts, the number of organisms left after the washing is likely to be high and, moreover, growth is greatly favored by the nutrients thus provided. In addition to causing contamination of dairy products, growth of organisms in utensils is objectionable because of the odors developed and the tendency of some of the products formed to accelerate the action on certain metals.

Strainers and separators. Contamination of milk from strainers and separators can be just as important as that from utensils. Metal strainers ordinarily are washed with the cans and pails and, accordingly, are subjected to the same general conditions. When a strainer cloth is used more than once and is left in a moist condition, there is a good opportunity for bacterial growth.

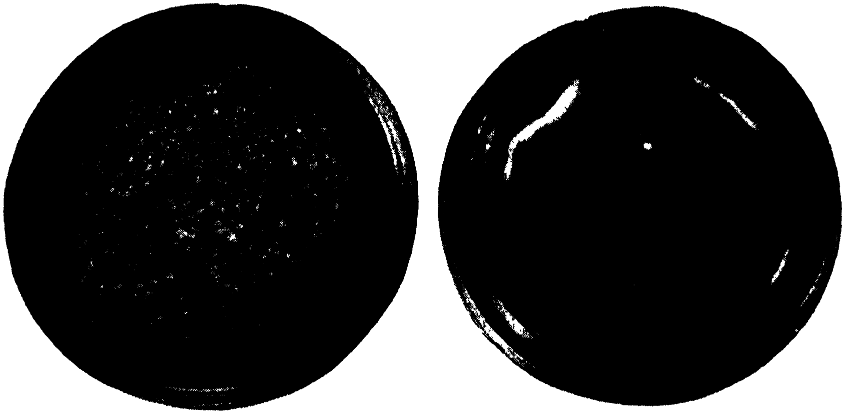
If separators are not washed every time they are used, milk and separator slime left in them afford an ideal food supply for bacteria so that, at suitable temperatures, growth is rapid; when the machines then are operated, large numbers of organisms are added to the skim milk and cream. Growth of organisms in unwashed separators, especially during the warm season, frequently results in undesirable odors which are taken up by the material run through the machines. The experienced cream judge often detects objectionable flavors that are recognized as coming from unclean separators.

Importance of an unwashed separator as a source of organisms in cream is shown by data obtained at the Iowa Agricultural Experiment Station. In each trial one-half of a lot of milk was separated with a machine that had stood without cleaning since the last period of use; the machine then was thoroughly washed and steamed, after which the remaining milk was separated. In various trials the bacterial contents of the cream obtained from the unwashed and washed separator, respectively, were 77,000,000 and 3,600,000 per milliliter; 46,000,000 and 8,450,000; 5,600,000 and 36,500; 101,000,000 and 1,005,000; 11,350,000 and 860,000; 113,000,000 and 960,000.

Species of organisms from utensils. Species of organisms added to milk as a result of contamination from utensils are varied. Organisms that grow well in milk commonly are prominent among them since, owing to the presence of milk solids, conditions for growth in utensils are similar to those in milk. This suggests that *S. lactis* may be added to milk from utensils and, under average farm conditions, pails and cans often are important sources of this species. In certain cases ropy milk organisms have come from utensils. Organisms which do not grow well in milk remain more or less inactive in utensils and then are added to the milk when the utensils are used. In attempts to reduce bacterial counts or control various fermentations, the possibility of contamination from utensils must be recognized.

Care of utensils. Since utensils may be responsible for addition of large numbers of bacteria to milk, adequate care is necessary in the production of milk with a low count. Such care consists of thorough washing, destruction of practically all the organisms present, and drying so that organisms will not have an opportunity to grow in the water and traces of nutrients present in undried utensils.

Washing of the utensils should begin with rinsing out of the milk with cold or only slightly warmed water; hot water often causes some of the milk constituents to stick to the utensils and these materials then are difficult to remove. After rinsing, the utensils should be scrubbed with hot water containing washing reagent. Use of soap is not advisable because it is more difficult to remove than washing reagents, and traces left may give milk a soapy taste. If the wash water is not reasonably clean it may be an important source of organisms. After proper washing, thorough rinsing again is necessary and this should be followed by steaming, scalding, or some comparable treatment. Unless a special method of drying is to be used, the metal should be heated so thoroughly that the water remaining after the utensils have been carefully drained will evaporate. Escape of the water vapor is greatly facilitated by leaving the utensils upright; when they are inverted on racks, the vapor rises and, since it cannot readily escape, it is condensed and much of the water remains. Utensils should not be dried with a cloth because of the possibility of adding large numbers of organisms. Inversion of the dry utensils in a clean atmosphere is an aid in limiting air contamination. Harding *et al.*[32] found that protection of thoroughly steamed milk pails against accidental contamination had a measurable effect in reducing the bacterial content of the milk.



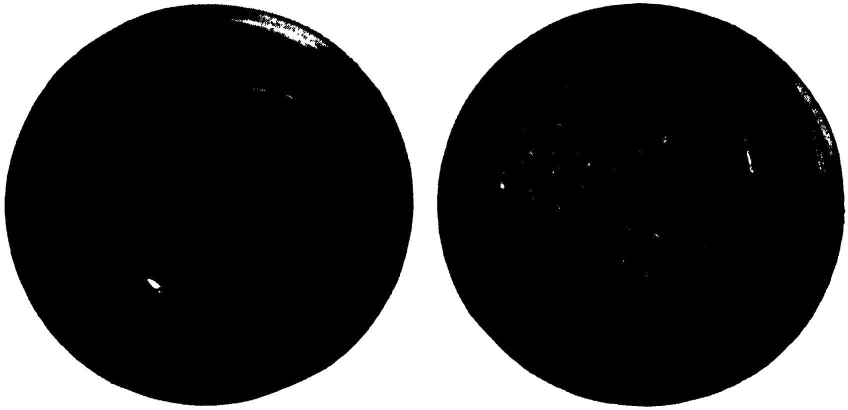
Agar disc from a washed but unsteamed can.

Agar disc from the same can after steaming.

FIG. 19. Influence of steaming on organisms in a milk can as shown by the agar disc method. Iowa Agr. Exp. Sta. Bul. 300.

Prucha and Harding[33] noted that rinsing cans removes some of the organisms mechanically and that, as the temperature is increased, destruction of organisms begins. Small amounts of water are cooled quickly when added to a can and quantities carrying sufficient heat to raise the temperature of the metal properly are necessary. Harding *et al.*[34] presented data indicating the necessity of using sufficient steam if the proper destruction of organisms during the steaming of cans is to be obtained. When dry cans filled with moist, warm air are closed, cooling may result in condensation of the moisture so that the cans then have water in them. This can be avoided by allowing the cans to cool slowly, or by blowing cool air in them, before covering. In steaming cans over a jet, sufficient time must elapse before the cans are covered if condensation of moisture is to be avoided.

Ayers and Mudge[35] considered the possibility of sterilizing utensils with hot air. They found that raising the temperature to 120 C (248 F) during a 4-min period destroyed a great many bacteria but did not give uniformly low results, whereas an added holding period of 2 min at 120 C increased the destruction. When a temperature of 140 C (284 F) was reached in 4 min, there was a high destruction of organisms and holding at this temperature increased it.



Agar disc from a freshly steamed can that was covered before the moisture could escape.

Agar disc from the same can after standing covered for 24 hr.

FIG. 20. Growth of organisms in a moist milk can as shown by the agar disc method. Iowa Agr. Exp. Sta. Bul. 300.

Utensils can be sterilized by putting them in a cabinet or container in which they can be subjected to heat. Where steam is available, a cabinet into which the steam can be turned is very useful. A metal container in which steam can be generated over a fire is also satisfactory; equipment of the latter type can be used to heat water for washing purposes as well as for sterilization.

Equipment that washes, steams, and dries cans is very satisfactory, if properly maintained and operated. Various dairy organizations have found that adequate maintenance requires frequent inspections by someone who understands the construction of the equipment. Carelessness in operation results in underheated cans, moist cans, etc. Cans returned to farms often are in such a condition that growth of organisms occurs in them and then there may be extensive contamination of the milk or cream for which they are used.

The presence of adhering milk solids affects the sterilization of utensils. Heat is transferred relatively slowly to the organisms imbedded in the solids. Organisms in fat may be protected additionally. From the standpoint of food sterilization, Yesair *et al.*[36] noted that moist cocci (staphylococci, streptococci, diplococci, etc.), or those dried over calcium chloride in vacuum, were low in heat resistance under moist conditions. Resistance was higher in moist fat. Resistance in dry fat was exceptionally high and approached that obtaining under conditions of dry sterilization. The mechanism of fat protection in foods appeared to rest on the localized absence of moisture.

Construction of utensils. Proper care of utensils is only possible when they are in a satisfactory mechanical condition. Rough areas and sharp depressions on the inner surface of a utensil make the complete removal of milk solids difficult and thus complicate the washing. Any seams that are present should be well filled with solder so as to avoid points at which milk solids can accumulate. Types of construction in which seams are avoided have a definite bacteriological significance.

Influence of sterile utensils on bacterial count. Influence of sterile utensils in reducing the bacterial content of milk is illustrated by the findings of Ayers *et al.*[37] in a comparison of sterile pails and cans with those in an unsterile condition. Throughout the trials the floors and cows were dirty, the manure being removed only weekly. Milk representing the unsterile utensils was obtained from July 22 to Aug. 14, inclusive; that representing the sterile utensils was obtained from Sept. 14 to Oct. 7, inclusive. With open pails, the average count when the utensils were not sterile was 497,653 per milliliter (26 samples), when the utensils were sterile it was 22,677 (36 samples); with small top pails the corresponding counts were 368,214 (28 samples) and 17,027 (36 samples). The data show that a greatly reduced bacterial count accompanied the sterilization of the utensils, even under the otherwise unsatisfactory conditions of production. Although the counts were lower with the small top pail than with the open pail, sterilization of the utensils was of much more importance than the type of pail.

Ayers *et al.*[37] made a more direct determination of the effect of sterile utensils on the bacterial count by obtaining data on milk from a sterilized, small top pail and from one that was unsterilized, the milk from two cows being secured with each pail. The cows were cleaned and bedded but the udders were not washed. Sixty samples from the sterile pail had an average bacterial content of 6,306 per milliliter with a maximum of 21,500, whereas 59 samples from the unsterilized pail averaged 73,308 and had a maximum of 284,000. In other trials the cows were dirty and the manure was removed only twice a week. Thirty samples from sterile utensils averaged 31,040 bacteria per milliliter; 50 samples from utensils that were washed directly after milking, but not sterilized, averaged 666,520; in 20 trials the utensils were held 8 hr before washing and the counts then averaged 1,667,000.

The following data reported by Lochhead [16] show the great effect of sterilization of small top pails on the bacterial count of milk:

EFFECT OF STERILIZATION OF SMALL TOP PAILS ON BACTERIAL COUNT OF MILK

	Bacteria per Milliliter of Milk
Pails cleaned with cold water	33,700
Pails rinsed with cold water, washed with hot water, but not sterilized	14,460
Pails sterilized after washing	3,200

The influence of unsterile utensils on the bacterial content of milk is extremely variable and depends on such factors as care used in the washing, heating incident to the washing, and opportunities for growth of organisms between washing and use. On farms where no attempt is made to give the utensils proper care, large numbers of organisms commonly come from them, especially during the warm months when there is rapid growth in the moisture present.

Contamination from milking machines. Introduction of milking machines provided an additional source of bacteria on the farms that is very serious unless suitable methods of treatment are employed. Early investigations of the bacteriological condition of machine-drawn milk indicated that the counts commonly were higher than those of hand-drawn milk. Harrison,[38] who carried out some of the first studies, found that 235 samples of machine-drawn milk contained 71,124 to 243,327 bacteria per milliliter, with most of the counts between 100,000 and 200,000, whereas 94 samples of hand-drawn milk varied from 645 to 63,422, with the numbers usually between 1,000 and 10,000.

Hastings and Hoffmann [39] reported results as follows: Of 150 samples of machine-drawn milk, 22 per cent contained less than 1,000 bacteria per milliliter, 55.3 per cent contained between 1,000 and 5,000, and 22.7 per cent contained more than 5,000; of 136 samples of hand-drawn milk, 19.8 per cent contained less than 1,000 per milliliter, 44.1 per cent contained between 1,000 and 5,000, and 36.1 per cent contained more than 5,000. Edwards[40] found counts from 203,000 to 1,208,000 per milliliter with 7 samples of machine-drawn milk, whereas 6 counts on hand-drawn milk ranged from 3,200 to 68,900; when the rubber parts and cover of the machine were boiled once a week the count was somewhat reduced and, with special precautions in the preparation of the machine, 2 samples were obtained with counts of 1,407 and 1,776 per milliliter. Meek[41] noted averages for a considerable number of samples as follows: milk from individual cows, hand-drawn 21,115 per milliliter; milk from individual cows, machine-drawn 47,870; mixed milk, hand-drawn 26,895; mixed milk, machine-drawn 83,143. Harding *et al.*[42] found that 20 samples of machine-drawn milk averaged 692,542 per milliliter, and an equal number of hand-drawn samples averaged 16,643; the cows supplying the samples were milked alternately by hand and machine. Larsen *et al.*,[43] in 38 comparisons, noted that the bacterial content of the hand-drawn milk averaged 10,739 per milliliter; that of the machine-drawn milk averaged 509,285.

It was early assumed that the high bacterial counts so often obtained on machine-drawn milk were due to contamination from the machine itself and this was readily proved by the reduction in counts when the machines were given suitable treatment. The more or less complicated construction makes cleaning difficult on the ordinary farm unless special methods are used, and carelessness is quickly reflected in the bacterial content of the milk. Various methods of adequately treating milking machines have been developed and the primary problem is to have one of them adopted and followed by each producer.

Information from various sources indicates that much machine-drawn milk is of very poor quality. When high bacterial counts are found on the milk from a certain farm, milking machines commonly are suspected. It has been noted in various dairy sections that installation of a milking machine on a farm often results in a decrease in the general quality of milk or cream produced. In such cases the primary difficulty is that the users of the machines do not recognize that adequate methods of care are essential. That very low-count milk can be produced with machine milking has been repeatedly shown. Milk of the finest possible bacteriological quality often is machine drawn. All that is needed is adequate care of the machines, so that they will not be significant sources of organisms.

Sources of the bacteria in machine-drawn milk. Ruehle *et al.*[44] found that the chief sources of bacteria, when large numbers were added to milk by a milking machine, were the teat cups and rubber tubes, although the pails were important in some cases. Air contamination was unimportant, as would be expected from information available on the bacterial content of stable air. Dropping the teat cups on the floor caused relatively large amounts of dirt in the pails, but analyses of sterile water drawn through the machines did not show the high bacterial counts that might be expected, since the values were not in excess of 25,000 per milliliter.

Importance of condensation water from the vacuum line as a source of bacteria in machine-drawn milk is evident from the studies of Stocking and Mason [45] and of Moak.[46] Investigations of Breed and Bright,[47] reported in 1921, indicated that many of the commonly used mechanical milkers were not then equipped with check valves that closed perfectly enough to prevent entirely the return of condensation material. Although this contamination did not appear to be extensive in any of the machines tested, it was of sufficient importance to be of significance in production of high-grade milk and to make it difficult to obtain premiums for low counts. A visit to 50 dairies in the New York districts supplying high-grade milk showed that nothing else was so generally neglected in the care of milking machines as cleaning the check valves, stanchion hose, and vacuum lines.

Condensation water in vacuum lines provides material in which organisms may develop rapidly, especially during the warm months. In many cases the odor is indicative of extensive bacterial action. Dirty material returning from the vacuum line may interfere with the proper closing of the check valves.

Macy [48] listed the chief sources of the contamination of machine-drawn milk, in the order of their importance, as follows: (1) teat cups, (2) rubber tubes, (3) pails and heads, (4) condensation water from the vacuum line, (5) stable air, (6) filth on teat cups if dropped on the floor.

Bacterial flora of milking machines. Hastings and Hoffmann [39] compared the types of bacteria in machine- and hand-drawn milk by means of litmus lactose gelatin plates and noted no marked difference. Robertson [49] studied the organisms in milking machines; samples were taken from various parts of the machines, including pipe lines and stanchion hose, by rinsing with sterile water, plating this, and then isolating the colonies which were predominant on the plates. The results obtained indicate that certain relationships exist between the bacterial flora and the care given the machines. Gram-negative rods and *S. lactis* were found quite regularly but were more prominent as the condition of the machines became less satisfactory. White, Gram-positive cocci were the most common type living in the machines when a brine hypochlorite solution was used for treatment. Alkali-forming rods appeared to be associated with holding the tubes in cold water or old sterilizing solutions of inadequate strength. Spore formers were rarely isolated and only a few *Escherichia-Aerobacter* cultures were obtained. Molds, which were primarily *O. lactis,* and yeasts were found in accumulations of old milk in various parts of the machines. Lochhead and Johns[50] noted no significant differences in the bacterial flora when low-count milk drawn by machines treated with hot water or chemicals was compared with hand-drawn milk of similar grade. With inadequate washing of the milk tube system there was an increase in the proportion of alkaline and proteolyzing types and a decrease in the proportion of inert types.

Care of milking machines. The care of milking machines involves two distinct procedures, (1) washing, and (2) sterilization; sterilization refers to the practical sterility advisable with dairy equipment rather than absolute sterility.

A machine should be washed as soon as the milking is completed by drawing through each unit a pail of cold water, then a pail of hot water containing washing reagent, and finally a rinse of hot water. The teat cups should be doused in and out of the water so that water and air alternately are drawn through the unit, and the removal of milk solids aided in this way. The outside of the teat cups and tubes should then be cleaned. Sterilization of the teat cups and tubes is carried out with either a chemical solution or application of heat; either method is satisfactory. Chemical treatment is now accepted by regulatory officials who at one time opposed it. Occasional dismantling of the teat cup assembly is advisable.

Chemical treatment. With chemical treatment, the teat cups and tubes are immersed in the sterilizing solution or suspended in a rack equipped with a container of sterilizing solution that permits filling the cups and tubes. Solutions of chlorine compounds, especially hypochlorites, have been widely used with milking machine parts; usually from 100 to 200 ppm available chlorine are recommended. Parfitt[51] suggested use of dilute sodium hydroxide (0.3 to 0.5%) and noted that it is cheap, readily available, relatively stable, and prevents formation of a precipitate on the rubber parts. Fay *et al.*[52] reported that milking machine tubes and teat cups can be sterilized with chlorine solutions testing 100 ppm available chlorine or 0.3 per cent sodium hydroxide, using the rack method; with immersion the chlorine solutions were less satisfactory than the sodium hydroxide. Many additional investigations indicate the value of sodium hydroxide in the control of bacteria in milking machines.

With the immersion method a large earthenware jar practically full of the sterilizing solution commonly is used. The clean teat cups and tubes are immersed in this slowly so that the air is driven out and the solution is in contact with all the parts. When needed, the cups and tubes are removed and clean, cold water drawn through them to remove the sterilizing solution. The solution must be kept up to proper strength at all times to ensure destruction of organisms, and should be renewed occasionally.

With the rack method the solution is allowed to flow slowly into the cups and tubes and fill them completely. When the machine is to be used these parts are emptied and rinsed with clean, cold water. The small amount of solution needed with this method makes it possible to have a fresh supply for each sterilization.

Other chemicals have been suggested for the treatment of milking machine tubes and teat cups. Rogers and Evans [53] suggested trisodium phosphate and a small amount of sodium chromate in water; poor results were obtained part of the time due to a gradual build up of spores in the solution. Mallman *et al.*[54] reported that a detergent mixture containing sodium metaphosphate prevented formation of milkstone on both metal and rubber parts of milking machines; use of machines kept free of milkstone made it possible to produce a lower-count milk than was produced with machines containing milkstone. Wetting agents also are being suggested for use in washing milking machines; after the washing, boiling water is drawn through the machine and the teat assembly drained and allowed to dry.

Effectiveness of sodium hydroxide solution in the care of milking machines has been thoroughly established. Apparently, the machine parts need be exposed to the solution only a relatively short time to control bacterial growth in them. Johns [55] has emphasized that with a 5-min exposure milk having relatively low bacterial counts was produced over an extended period. The procedure avoids the usual difficulties with freezing during cold weather. Because of the effectiveness of the sodium hydroxide solution, milk with relatively low bacterial counts has been obtained by simply rinsing the machine with cold water before using the solution.[56]

Dismantling of the teat cup assembly is necessary but there is a difference of opinion as to how often it should be done. At one time daily dismantling was suggested. More recently less frequent dismantling has been advocated; in general, bacterial counts on the milk have not been increased by this. The Arizona Agricultural Experiment Station [57] compared the bacterial counts of milk produced with weekly dismantling of the milking machine and with daily dismantling. The results were as follows:

COMPARISON OF BACTERIAL COUNTS OF MILK WITH WEEKLY AND DAILY DISMANTLING OF MILKING MACHINE

	Bacteria per Milliliter	
Period	*Weekly Dismantling*	*Daily Dismantling*
Dec., Jan., Feb.	8,170	24,600
Mar., Apr., May	7,961	16,600
June, July, Aug.	5,264	9,060
Sept., Oct., Nov.	4,100	13,600
Average all counts for 2 yr	6,200	18,024

Heat-treatment. Heat-treatment usually consists of immersing the teat cups and tubes in water and raising the temperature. The value of this procedure was pointed out by Hart and Stabler [58] in California, in 1920; they concluded that heat sterilization is the only way to treat milking machine rubber parts successfully under ordinary ranch conditions. The heat has an effect on the life of the rubber parts but, if care is used to prevent excessive temperatures, this is not serious. A temperature of 71.1 to 76.7 C (160 to 170 F) for about 30 min appears to give satisfactory destruction of organisms. The parts may be left in the water until the next milking or various other procedures may be followed. Burgwald [59] found that heating a unit in water at 71.1 to 75 C (160 to 167 F) for 20 to 35 min, removing it to a refrigerator, and keeping it there between milkings increased the life of the rubber parts over those left in the hot water, and resulted in counts which were only slightly higher than those of milk drawn with equipment left in the water. Holding the heat-treated unit in a weak chlorine solution between milkings gave counts that were still lower than those obtained with the unit held in the water. Burgwald also compared temperatures of 62.8 to 65.6 C (145 to 150 F) and 71.1 to 73.9 C (160 to 165 F), the units being left in the water between milkings. With the lower temperatures the life of the rubber parts was greater than with the higher temperatures but the bacterial counts were not as low; 142 samples showed a range of counts from 3,000 to 51,000 per milliliter, with an average of 11,930, whereas 40 counts on milk drawn following the use of the higher temperatures ranged from 700 to 6,000 and averaged 2,520. A method of holding the unit in water at 71.1 to 73.9 C for 20 to 45 min and then hanging it in a warm room also was used; 154 samples of milk obtained in this way varied from 1,100 to 24,000 per milliliter and averaged 5,540. With heat-treatment there was a tendency for the rubber tubing to stick to the metal parts unless the units were taken apart rather frequently.

Fisher and White[60] found that sterilization with steam was very destructive of rubber parts, but with hot-water treatment at 93.3 C (200 F), or above, for 0.5 hr damage to the rubber was not prohibitive and counts on the milk were uniformly low.

Henderson *et al.*[61] found that the heat-treatment of milking machine rubber parts, using water at 85 C (185 F) and leaving the parts in the water for 20 min, was the most satisfactory method of heat sterilization used. Life of the rubber parts was not materially less than with 76.7 C (170 F) for 20 min. When the parts were heated to 76.7 C and held for 20 min, or when they were left in the water to cool gradually until the next milking, there was evidence of growth of thermophilic bacteria. Hypochlorite solution containing 200 ppm chlorine, or sodium hydroxide in a concentration of 0.3 or 0.5 per cent, gave bacterial counts somewhat higher than sterilization at 85 C but was effective in controlling thermophilic organisms. With different methods of heat sterilization life of the teat cup liners was approximately the same, but was 33 per cent less than with chlorine or sodium hydroxide.

Holding in cold water. Holding the washed teat cups and tubes in a container into which cold water is allowed to run continuously has been tried as a means of keeping milking machines in a satisfactory condition, bacteriologically. Ruehle *et al.*[44] found that as long as the water was below 18 C (64.4 F) it effectively controlled bacterial growth, but that as soon as it reached this temperature a change occurred and much larger counts appeared. Robertson *et al.*[62] noted that tubes held in cold running water eventually became coated with large numbers of water bacteria, even when the temperature was lower than 14.4 C (58 F); accordingly, they did not regard it as a satisfactory method of caring for tubes. Fisher and White[60] reported that a stream of water, at temperatures well below 12.8 C (55 F), running through the units between milkings, was a convenient and reliable means of producing good-quality milk but that with high water temperatures the method failed. Macy[48] noted that some dairymen have obtained good results by placing teat cups and milk tubes in running water when the temperature is below 10 C (50 F).

Milking machine pails, etc. Milking machine pails, lids, etc., may be a source of organisms, just as other farm utensils, and should be given adequate treatment.

3. Coat of the Animal

Contamination of milk from the coat of the producing animal involves mainly the dirt falling into the milk during the milking process. This includes manure, bedding, soil, epithelial cells, etc.; it carries organisms, often in large numbers, and these are readily distributed through the milk. The dirt ordinarily present in milk very largely gains entrance during the milking process, although windblown dust, dirt, and debris, material from the hands and clothing of the milker, and material from the utensils may be included; with milking machines, manure, bedding, etc., occasionally are sucked from the floor by accident.

Amount of dirt in milk. The amount of dirt in milk can be determined on a comparative basis by use of cotton discs, or on an actual weight basis by collecting it through sedimentation and/or centrifuging; none of the methods recover all the dirt present. Harding and Prucha[63] found that when the cows were unusually dirty and were milked into an open pail, dirt in the unstrained milk amounted to 10.8 mg per quart, whereas with methods comparable to those used in the better dairies the amount was about 2.5 mg. Under the former condition the increase in bacterial content of the milk due to the dirt was about 17,000 per milliliter. The investigators found the bacterial content of dirt from the coat of a clean cow to be 17,814,000 per gram, whereas that from the coats of four dirty cows varied from 184,300,000 to 4,592,000,000; considerable variation in the bacterial content is to be expected because of differences in the nature of the dirt.

Bacteria in cow manure. Prucha *et al.*[64] collected 27 samples of fresh cow feces, held them at about 23.9 C (75 F) and a relative humidity between 20 and 40, and determined the numbers of bacteria by the plate method at intervals during 3 weeks. The bacterial content of the fresh feces ranged from 434,000 to 73,200,000 per gram of dry matter and averaged 8,486,000; the moisture content varied from 78.4 to 87.6 per cent and averaged 83.6. In all the samples there was multiplication of the bacteria present. The rate of increase was very rapid during the first 2 days, after which it became slower but continued for several days until a maximum was reached. A slow decrease then began. The maximum number in nearly every sample reached several billion per gram of dry matter; in six it was between 10 and 11 billion; and in only one was it less than 1 billion. Many of the organisms present were gas formers.

Allen,[65] using the plate method, found that 50 samples of fresh cow feces contained 160,000 to 634,000,000 organisms per gram and 49 samples which had been dried on porous plates at 37 C (98.6 F) for 48 hr contained 500,000,000 to 16,800,000,000. Representative colonies from plates poured with the fresh feces were inoculated into litmus milk and the changes produced showed that a variety of organisms was present.

Solubility of cow manure in milk. Taylor [66] investigated the solubility of cow manure in milk and summarized the results as follows:

Average Solubility of Cow Manure in Milk

	Percentage Moisture in Manure	Percentage Solids in Manure	
		Soluble in Milk	*Insoluble in Milk*
Fresh manure	82.76	0.96	16.25
Air-dried manure	5.74	5.22	89.04

Manure was found to be less soluble in milk than in water. The results also indicated that 91 per cent of the manure present in bottled milk will be visible at the bottom of the bottle.

By mixing cow manure (1 sample air dry and 1 sample moist) with milk, Duggan [67] found that the visible filth filtered from milk on sediment pads or filter cloths represented approximately 12 per cent of the original wet manure incorporated in the milk; the rest of the manure remained in solution.

Effect of dirt on flavor of milk. Marquardt [68] noted that the flavor of milk improved with an increase in the percentage of clean milk deliveries. The flavor of clean milk deteriorated more slowly than that of unclean milk when held 4 to 6 hr at 21.1 C (70 F).

Detection of manure fragments in milk. Bits of plant tissue may be in milk as a result of contamination with either dust and dirt or manure. From the standpoint of milk control, the latter source is much the more objectionable. Staining procedures [69] have been developed for detection of manure fragments in dairy products so that contamination with manure cannot be ascribed to other materials.

Species of organisms from coats of cows. The species of organisms entering milk from the coats of the cows are, in general, those found in manure, soil, feeds, and bedding. Many of them are very objectionable because of the rapid changes which they bring about in milk; *Escherichia-Aerobacter* types are especially undesirable.

FIG. 21. Cows and stable in good condition for production of low-count milk. Courtesy M. P. Baker.

Influence of clean cows on bacterial count. Control of contamination from the coats of the cows requires that the animals be kept reasonably clean. This involves frequent brushing and, in the case of farms producing a special grade of milk, washing or wiping the udders and flanks; occasionally, more complete washing of the animals is practiced. Method of bedding and type of stanchion are important factors in preventing the coats of the animals from accumulating manure.

Stocking[14] studied the effect of wiping the udders and flanks with a damp cloth on the bacterial content of milk, using two groups of five cows each and milking one group with such wiping and the other without; the groups were alternated to eliminate variations in contamination from the interiors of the udders. A small top pail was used and the milk from the five cows in a group was mixed before sampling. The average numbers of total, acid-producing, and liquefying bacteria per milliliter in the milk were 7,058, 3,554, and 81, respectively, when the udders and flanks were not wiped; and 716, 185, and 47 when the udders and flanks were wiped. In all but one of the 13 comparisons milk from the wiped animals had the lower count; often the difference was small but in some instances it was rather large.

Byers and Ewalt[70] used 7 cows to determine the comparative values of water and chlorine solution (150 ppm) for washing udders and rinsing milking machine teat cups. With 490 samples each, the average bacterial count on the milk with water was 2,171 per milliliter and with chlorine it was 1,428.

Data presented by Ayers *et al.*[37] show the influence of cleaning the cows and floors on the bacterial content of milk; the main effect of clean floors undoubtedly was in keeping the animals clean. Samples from the dirty animals were obtained from Apr. 11 to May 6, inclusive, and those from the clean animals from June 5 to June 13, inclusive. The summarized results are as follows:

EFFECT OF CONDITION OF THE COWS ON BACTERIAL CONTENT OF MILK

	Cows and Floor Dirty, Manure Removed Weekly, Utensils Sterile		Cows and Floor Clean, Manure Removed Daily, Udders and Teats Washed, Utensils Sterile	
	Number of Samples	*Average Number Bacteria per Milliliter*	*Number of Samples*	*Average Number Bacteria per Milliliter*
Open pail	41	86,212	15	4,947
Small top pail	41	24,439	15	2,667

The reduction due to cleaning of the animals and washing of the udders and teats was pronounced. In additional investigations Ayers *et al.*[37] studied the effect of washing the udders and teats on the bacterial content of milk by having four cows cleaned and bedded, the floor clean, and the manure removed daily, and then milking two of the cows after the udders and teats had been washed, while the other two were milked without such washing; the utensils employed were sterile. The 65 samples from the washed udders averaged 2,154 per milliliter with a maximum of 5,400; the 65 samples from the unwashed udders averaged 4,524 per milliliter with a maximum of 20,400. From the data it is evident that even when the animals are in such a condition that contamination from the coats is slight, washing of the udders and teats brings about a reduction in bacterial content of the milk.

Small top pails. Small top or sanitary milk pails are intended to reduce the area through which dust and dirt can fall into the milk and, in this way, to decrease contamination from the coat of the animal and the air. They are very helpful in production of clean milk and are widely employed. A number of types have been devised but a simple construction is advisable so that the pails can be washed and used without inconvenience.

Stocking[71] noted large reductions in the bacterial content of milk as a result of using small top pails; under dirty stable conditions the counts obtained with one type were only 3 per cent as high as those obtained with an open pail. Harding *et al.*[72] found that more than half of the contamination of milk could be prevented by small top pails; six types of pails studied gave reductions from 48.4 to 70.1 per cent.

Ayers *et al.*[37] compared the counts obtained with open and small top pails under different conditions of production; results are summarized in the following table:

INFLUENCE OF SMALL TOP PAILS ON BACTERIAL CONTENT OF MILK UNDER VARIOUS CONDITIONS

	Open Pails		Small Top Pails	
Conditions of Production	*Number of Samples*	*Average Number of Bacteria per Milliliter*	*Number of Samples*	*Average Number of Bacteria per Milliliter*
Cows and floor dirty, manure removed weekly, utensils not sterile	26	497,653	28	368,214
Cows and floor dirty, manure removed weekly, utensils sterile	36	22,677	36	17,027
Cows and floor dirty, manure removed twice a week, udders and teats washed, utensils sterile	18	6,166	23	2,886
Cows and floor dirty, manure removed weekly, utensils sterile	41	86,212	41	24,439
Cows and floor dirty, manure removed weekly, utensils not sterile	36	153,905	36	114,497
Cows and floor clean, manure removed daily, udders and teats washed, utensils sterile	15	4,947	15	2,667

Under all the conditions studied, milk drawn in small top pails had a lower bacterial count than that drawn in open pails and, commonly, the difference was pronounced.

The following data reported by Lochhead [16] also show the effect of small top pails in reducing the bacterial count of milk under various conditions:

EFFECT OF SMALL TOP PAILS IN REDUCING BACTERIAL CONTENT OF MILK UNDER VARIOUS CONDITIONS

	Bacteria per Milliliter of Milk		
	Open Pail	*Small Top Pail*	*Decrease*
Generally sanitary, careful methods	4,560	3,200	1,360
Pails clean, animals and stable clean, milker careless	10,490	9,150	1,340
Pails clean, cows milked under careful conditions, stable clean, animals not regularly cleaned	37,670	12,000	25,670
Generally careless conditions	397,600	192,500	205,100

Reduction in the bacterial content of milk as a result of use of small top pails is extremely variable and depends especially on such factors as condition of the coat of the cow and amount of dust in the air. Under unsatisfactory conditions of production the number of organisms kept out of the milk per milliliter is larger than under good conditions, but in both cases reduction on a percentage basis is considerable.

4. MILKER

The milker may add organisms to the milk from the hands and clothing, and also by coughing, sneezing, or speaking over the utensils or milk. Organisms from the latter sources are especially objectionable because of the possibility of pathogens being included.

During milking, organisms are dislodged from the hands and often fall into the milk. The method of milking with the hands wet is very insanitary because some of the liquid, carrying dirt and bacteria from the hands and the outside of the udder and teats, drips into the milk. Dirt often falls into the milk from dirty clothing, especially when a pail is carried so that the top brushes against it.

Extent of contamination from the milker is largely determined by the general habits of the individual. A milker should be neat and clean, and should appreciate the importance of suitable methods in the handling of materials intended for human consumption. Clean hands add fewer organisms than dirty ones, and in some dairies interested in production of milk of an exceptionally high sanitary quality the hands are washed before each cow is milked. The milk should not touch the hands since, if it does, it tends to wash off dirt and bacteria that may be present. Clean clothing is very desirable because the dirt falling from unclean garments may get into the milk. The principal advantage of wearing white suits when working with dairy products is that they quickly show an accumulation of dirt. Such practices as working in a hot, dusty field and then milking without changing the clothing or washing the hands are very objectionable and must be eliminated if the confidence of the consumers of milk is to be retained.

5. MISCELLANEOUS SOURCES

Miscellaneous sources from which milk is contaminated include those that are involved with certain lots of milk but not with others: examples are flies, cooling water splashing into the milk, visitors coming in contact with the utensils or milk, and dropping of a utensil which then is used without proper treatment. In some instances the miscellaneous sources of contamination are of considerable significance; flies illustrate the possibilities.

Flies are responsible for contamination of milk, either indirectly through contaminating utensils or directly by falling into it. In a series of experiments reported by Esten and Mason [73] in which 414 flies were examined, the numbers of bacteria present on the body of a fly varied from 550 to 6,600,000 and averaged 1,222,570. Armstrong [74] also reported counts of the organisms that could be washed from the bodies of flies; seven flies caught in clean houses carried from 600 to 75,000 bacteria per fly and averaged 13,986, whereas six flies caught in dirty houses carried from 1,050 to 4,000,000 and averaged 1,106,017. Because flies so commonly alight on fecal material, organisms on their bodies include various species, especially *Escherichia-Aerobacter* forms, which produce changes in milk and cream rapidly. When human fecal material is exposed, there is the possibility of typhoid organisms and other intestinal pathogens being carried to milk.

Importance of contamination from external sources. The contamination that milk receives from external sources ordinarily is the important contamination to which it is subjected on farms. The interior of the udder usually adds only small numbers of organisms and these produce slow changes in milk, but the external sources often add large numbers, many of which cause rapid changes in milk. Extent of the contamination from external sources on various farms depends on the conditions prevailing. Ayers *et al.*[37] studied 249 samples of fresh milk from 12 farms before it was strained or cooled. The night milk averaged 115,135 per milliliter and the morning milk 180,696; the average for all was 135,146. A comparison of these counts with those obtained on milk drawn aseptically indicates that the external contamination may involve large numbers of organisms.

Important factors in producing low-count milk. Factors of importance in producing milk with a low bacterial count are sterile utensils, particularly milking machines, reasonably clean cows, and small top pails; these form the basis for any contemplated improvement in the sanitary quality of milk. None of the factors involve either an unreasonable effort or prohibitive expense, but do require an understanding of the care necessary in production of a food material, particularly one obtained under the conditions existing with milk. The incentive to produce milk of good sanitary quality that is provided by certain organizations in the form of a bonus for milk having low bacterial counts has shown what can be accomplished under ordinary conditions.

Ayers *et al.*[37] studied the effect of sterile utensils and small top pails in reducing the bacterial content of milk or cream on a number of farms during the summer when the cows were on pasture most of the time and their coats practically free of visible dirt; counts first were obtained for a considerable period on milk produced in the ordinary way and then on that produced with sterile, small top pails. Results are given in the following summary:

INFLUENCE OF STERILE, SMALL TOP PAILS ON BACTERIAL CONTENT OF MILK WHEN THE COWS WERE REASONABLY CLEAN

	Open Pails, Utensils Not Sterilized		Small Top Pails, Utensils Sterile	
Farm	*Number Samples*	*Average Number Bacteria per Milliliter*	*Number Samples*	*Average Number Bacteria per Milliliter*
1	19	116,384	16	10,637
20	18	15,050	16	4,656
27	19	186,995	16	3,606
29	19	77,095	16	1,962
43	18	34,861	16	2,150
178 *	16	49,181	16	3,025

* Cream produced, separator parts sterilized.

North[75] presented data illustrating the effect of sanitary methods in decreasing the bacterial content of milk produced on Pennsylvania farms. Milk drawn by the usual milkers was first studied and then milkers experienced in proper methods were brought to the farms. The imported milkers employed the old equipment, except that they used small top pails, and were on the farms not more than 2 hr. Representative counts on the milk obtained by the usual milkers and the experienced milkers, respectively, are as follows: farm *A,* 1,830,000 and 3,300 per milliliter; farm *B,* 1,520,000 and 3,100; farm *C,* 4,830,000 and 4,600; farm *F,* 3,600,000 and 61,000; farm *G,* 60,000 and 800; farm *J,* 500,000 and 5,600. The decreases in bacterial counts that resulted from use of satisfactory methods are very striking.

Results showing the effect of various factors in reducing the bacterial count of milk were reported by Lochhead,[16] as follows:

EFFECT OF VARIOUS CONDITIONS IN REDUCING BACTERIAL CONTENT OF MILK

	Bacteria per Milliliter of Milk
(a) Generally careless conditions throughout	398,000
(b) Careless conditions except that cows were brushed daily, without removing possibility of contact with manure	316,500
(c) Careless conditions except that care was taken to remove manure from contact with cows, cows not brushed	69,200
(d) Careless conditions except that manure was removed and cows brushed daily	48,800
(e) Like (d) but care exercised by the milker	27,600
(f) Like (e) but care given the milk pail	3,200

Results that have been obtained under widely varying conditions indicate that anyone who will use reasonable care can produce milk with a fairly low bacterial count. Elaborate equipment is not needed but an understanding of methods of preventing contamination from the important sources is very necessary. Suitable methods of production, combined with prompt cooling to low temperatures, ensure the delivery of milk which will have a low bacterial count and which, accordingly, will be free of abnormal flavors of bacterial origin and will keep satisfactorily.

General conditions. Certain areas definitely are better adapted than others to production of clean milk with low bacterial counts. In areas having long periods when the animals are in the stable and/or long periods of muddy conditions, it is difficult to keep the coats of the animals clean without special procedures that often are not applied to all the milk, especially that for manufacturing purposes. As a result there commonly is considerable sediment in the milk and the bacterial counts are relatively high. When conditions are such that the animals are in the stable only for milking and the soil is sand and gravel, with the rain water quickly being carried away, it is not so difficult to keep the animals clean. Accordingly, there is relatively little sediment in the milk and the bacterial counts are comparatively low, unless there is serious contamination from some such source as utensils. With sand and gravel soil there is less dust in the air and less sediment due to windblown material. Generally speaking, because of the great influence of climatic conditions, etc., contamination from the coat of the cow is the most difficult to control.

Milking rooms or parlors are a definite aid in production of clean, low-count milk. With them it is easier to observe the various precautions that are necessary to produce milk under good conditions.

Influence of the stable on milk contamination. At one time the stable was thought to be an important factor in production of milk low in bacteria and often a poor stable has been given as an excuse for a high count on milk. Investigations have shown, however, that milk of high sanitary quality can be produced in a stable of very poor type.

North [28] studied, for 1 yr, the sanitary quality of the milk produced in two barns of very different construction and surroundings, by running counts at least three times a week and often daily. In both barns suitable methods were used but with the old barn the cow yard, lighting, ventilation, floors, walls, and ceiling were in a very unfavorable condition. The bacterial content of the milk from the sanitary barn averaged 1,097 per milliliter with monthly averages from 287 to 3,300, whereas the milk from the old barn averaged 3,102 and the monthly averages were from 331 to 10,200. The results show that milk with a very low bacterial count can be produced in a stable that would ordinarily be considered unsatisfactory.

An interesting observation was made in connection with the cream improvement work carried out among the patrons of an Iowa creamery; the cream was not examined for bacterial content but was judged on the basis of flavor. One patron, whose cream was of the very highest quality, was found to be using a stable consisting of a pile of straw thrown over a framework of poles that, ordinarily, would be considered a poor place for production of high-grade milk or cream.

Harding *et al.*[32] studied the influence of different conditions of the walls and ceiling of a barn on the bacterial content of the milk, using 6 milkings from each of 6 cows for each condition; the bacterial content of the milk before plastering the barn averaged 775 per milliliter, that after plastering averaged 889, and that after painting the walls averaged 656. Influence of whitewashing also was studied with 20 samples from each of 3 cows, both before and after whitewashing; milk obtained before the stable treatment averaged 953 per milliliter and that obtained after the treatment averaged 1,193.

Although milk of low bacterial content can be produced in a poorly constructed stable, it is easier to obtain milk of good sanitary quality in satisfactory than in unsatisfactory surroundings and, for this reason, regulations covering milk production commonly include requirements with reference to the stable. A suitable building and good equipment are a distinct advantage in the proper handling of any dairy product and, moreover, the consumer expects all food products to be handled under clean conditions.

Influence of clipping the udder and flanks. The influence of clipping the udder and flanks of the cow on the bacterial content of milk appears to be negligible. Harding *et al.*,[32] using 2 cows and a number of milkings from each, found that the bacterial content of the milk before clipping the udders and flanks was 231 per milliliter; after clipping it was 331. In another trial, involving 22 samples from each of 4 cows before clipping and 24 samples from each after clipping the udders, flanks, and tails, the average count before clipping was 204 per milliliter; after clipping it was 320. Under certain conditions clipped animals are more easily kept clean than unclipped ones so that from this standpoint there may be an advantage in the clipping.

CONTAMINATION SUBSEQUENT TO PRODUCTION

Contamination of milk subsequent to production may be as extensive as that occurring on the producing farms. In dairy plants the use of equipment that is not in a satisfactory condition, bacteriologically, is especially serious and may add many organisms to milk and cream. Vats, coolers, pumps, bottle fillers, etc., all present the same general problems as the utensils on farms and contamination from them often is extensive because the construction complicates the cleaning. Many studies, especially line tests in which a series of samples are taken during the processing, show how extensive contamination of milk in plants can be. In many instances milk has been properly pasteurized and then heavily contaminated during the cooling, bottling, and handling.

Coolers. Coolers often add considerable numbers of organisms to milk. Judkins and Downs[76] found the average bacterial content of 6 lots of milk after pasteurization in a vat was 439.5 per milliliter, with a minimum of 45 and a maximum of 965, whereas after running over a cooler the average count was 4,104, the minimum 1,000, and the maximum 9,580. Smith[30] reported the results of counts made at a milk plant over a period of 5 days; the average count on the pasteurized milk was 6,240 per milliliter; that on the milk from the cooler was 8,452.

Bottle fillers. Bottle fillers are one of the most important sources of bacteria in milk plants. Prucha *et al.*[27] found that when a bottle filler which had stood for 20 hr after washing was not steamed before use there was a conspicuous increase in the bacterial content of the bottled milk; in ten trials in which the maximum count for the milk from the pasteurizer was 203 and the average 84 per milliliter, the maximum for the first bottle through the filler in each trial was 209,600 and the average 96,900. Use of the filler gradually washed the organisms from the machine so that the contamination became less as the run progressed.

Judkins and Downs,[76] with equipment which was rinsed with scalding water before the processing operation began, noted that in six trials the pasteurized milk in the vat averaged 439.5 bacteria per milliliter, the same milk at the bottler averaged 12,195, and in the bottles it averaged 48,033. Accordingly, the pump and piping taking the milk to the bottler added 11,755.5 per milliliter, and the bottling process added 35,838 more. Smith [29] carried out eight tests at a large milk plant and found the average count on the cooled milk was 2,038 per milliliter, whereas on the bottled milk it was 5,744; since the bottles at this plant were practically sterile the organisms must have come largely from the bottle filler. At a plant studied for 14 days the average count of the milk from the cooler was 21,750 per milliliter, that of the milk in the bottler was 28,350, and that of the bottled milk was 37,800. In another investigation Smith [30] found the average bacterial content of the milk from the cooler was 8,452 per milliliter, whereas the average count of the bottled milk was 15,592.

Homogenizers. Because of their construction, many homogenizers are difficult to maintain in a satisfactory bacteriological condition. Changes in construction, which have been prompted largely by sanitary considerations, have been very helpful in simplifying the care of the modern machines. The pressures under which the machines operate tend to force milk solids into crevices, etc., from which they are removed only with difficulty in the case of older models. Homogenizers have been considered most in connection with contamination of ice-cream mix but they also are important with homogenized milk and cream.

Study of the contamination from a homogenizer is complicated by the breaking up of clumps of organisms in the material run through it, so that an increase in the number of organisms, as determined by the plate method, may not be due entirely to addition of organisms. Running sterile water through the machine and then plating a sample of it may give a more accurate idea of the contamination than homogenization of cream. In some instances the contamination is very clearly shown by the presence of species in the homogenized material that are entirely lacking in the unhomogenized product.

James[77] found that the influence of the breaking up of clumps of organisms during homogenization on the plate count of cream was fairly constant during the handling of one lot, but that there was considerable variation between lots. Little difference was noted between pressures of 750 lb and 2,500 lb per square inch, which suggests that the lower pressure was sufficient to break up the clumps. This investigator made five runs with pasteurized skim milk, using a machine that was practically sterile, and noted that the counts remained fairly constant during one operation; rehomogenization had little influence on the count so that homogenizing once evidently was as effective in breaking up clumps as homogenizing twice. Comparative counts before and after homogenization varied from a ratio of 1 to 1.5 to a ratio of 1 to 4.6.

When the homogenizer is a significant source of organisms, the contamination becomes less during an operation, owing to the gradual washing out of organisms. In some instances the decrease is very rapid so that after the first few minutes only small numbers of organisms are added by the machine.

Influence of season on contamination from equipment. When equipment is not properly cleaned and dried, there commonly is a seasonal influence on the extent of contamination from it because of the effect of temperature on growth of organisms between the periods of use. During cold weather multiplication of organisms in moist equipment is relatively slight; during hot summer days it is certain to be extensive.

Care of dairy plant equipment. In the proper care of the equipment in dairy plants the same principles are employed that are used with utensils on farms. The washing involves rinsing with cold or warm water, scrubbing with hot water containing washing reagent, and then careful rinsing with hot water; when considerable fat is present, as is the case if the equipment has been used for cream, it is advisable to use warm water for the original rinse, rather than cold. Scrubbing is a step in the process that often is poorly done. In pasteurizing equipment, in the upper parts of coolers, and wherever milk or cream is exposed to high temperatures, there is more than the usual tendency for the milk solids to stick to the metal, and without thorough scrubbing the cleaning will be incomplete. If careless treatment continues there is a gradual accumulation of material that interferes with the heat transfer, develops objectionable flavors, and may make possible growth of organisms. The scrubbing should not be limited to surfaces that are easily reached but should include every part of the equipment.

The washed equipment should be steamed at once. Steaming must be thorough so that all the parts are well heated and, with complete drainage, the remaining moisture will evaporate. In some plants the steaming is so poorly done that the expected results cannot be obtained. When the steam can be largely confined, as in a vat or an internal cooler, the metal is easily heated through, but an exposed surface requires more attention, if the heating is to be properly done. It is not the presence of steam but the heat supplied by it that accomplishes the destruction of organisms, and raising the temperature of the equipment to the desired point can be accomplished only when the proper time factor is provided.

Although there are exceptions, with many pieces of equipment the proper steaming supplies heat enough to evaporate the water that cannot be drained away. Draining should be going on during the steaming, and as soon as the steaming is complete the water vapor should be permitted to escape quickly. If this is not done it is probable that moisture still will be present when the temperature has dropped to a point where evaporation is negligible, and there then will be an opportunity for growth of organisms in the equipment before the next period of use. In the cleaning of equipment that is complicated in construction disassembling during washing should be as complete as possible and the parts should be thoroughly scrubbed. Normally, a hot solution of washing reagent pumped through equipment cannot take the place of scrubbing, as is evident from the accumulations of milk solids and the odors so commonly noted when equipment is taken apart following periods of such treatment. After scrubbing, the parts should be rinsed, steamed, and dried as completely as possible. Whenever the steam must pass through an extended system of any sort, steaming should be continued until the entire metal surface has been thoroughly heated. In some instances in which the steaming of equipment apparently yielded unsatisfactory results from the bacteriological standpoint, more extended steaming brought about the desired destruction of organisms.

Attempts are being made to develop cleaning materials that can be pumped through pipe lines, and perhaps other equipment, and a satisfactory cleaning operation performed. These involve a number of special considerations. The pipe lines should be perfectly smooth on the inside with no breaks in which milk solids can accumulate; this presents a formidable fabrication problem. Also, brushes or similar devices to go through the lines, without stopping at angles, etc., are desirable. It is essential that the reagents used do not harm the metal excessively; some of the reagents that have been suggested have been very objectionable from this standpoint.

Equipment that has been properly cleaned and dried regularly accumulates organisms from the air. The numbers from this source vary widely, owing to differences in air conditions, but ordinarily they are not large. Because of the contamination of equipment from air and the possibility of growth of organisms in moisture that was unintentionally left in the equipment, it is a common practice to treat pipe lines, pumps, coolers, bottle fillers, etc., with hot water or steam just before use. Often hot water or steam is forced through a whole system that has been assembled for the handling of milk or cream. With this procedure it is essential that adequate amounts of hot water and steam be employed to ensure sufficient heating of the entire system. A germicidal rinse also can be used; it has the advantage of leaving the equipment relatively cool instead of very hot.

Improvement in construction of equipment. Various manufacturers have definitely recognized the importance of sanitation in the designing of dairy plant equipment and are building equipment that can be adequately and easily cleaned and sterilized. Some of the older equipment was so complicated and difficult to disassemble that proper care in plants was practically impossible.

Milk bottles. In some instances milk bottles have been sources of significant numbers of bacteria. Smith [29] studied 268 bottles, most of which were pints, and found that from <1 to 1,636 bacteria would have been added per milliliter of milk. Later, [30] 344 bottles from 32 plants were studied. Average contamination of milk under all conditions of washing would have equaled 288.3 organisms per milliliter; without a mechanical washer and steamer, contamination from 190 bottles would have averaged 458.2, with a range from <1 to 9,005; with a mechanical washer and steamer, contamination from 154 bottles would have averaged 78.8, with a range from <1 to 900.

In Great Britain, Hobbs and Wilson [78] examined 2,406 bottles from 105 bottle-washing plants of 26 types. The mean counts, reckoned as pints, at 22 and 37 C (71.6 and 98.6 F), respectively, of all bottles washed in large plants were 2,008 and 2,324; in small rotary plants, 7,982 and 7,389; in plants using steam sterilization, 23,671 and 9,704; and in plants with hand washing only, 96,065 and 37,818. Taking all large plants together, 55.5 per cent of the bottles had less than 600 colonies at 22 or 37 C and only 1.1 per cent had over 25,000; corresponding figures for small rotary plants were 49.0 and 6.4; for steam sterilization plants 50.0 and 23.5; and for hand washing 0.0 and 83.0. The authors noted that virtual sterility can be achieved either by using relatively strong caustic soda solutions at low temperatures or relatively weak solutions at high temperatures.

Use of chemical rinses on bottles just before they go to the bottle fillers is an added protection against excessive numbers of organisms in the bottles. However, with good washing and heating treatment such rinses probably are not necessary.

Bottle caps. Bottle caps constitute a possible source of milk contamination and when exposed to flies, dust, etc., may be of considerable importance. Regulations covering milk production commonly require adequate protection of bottle caps. Growth of organisms does not occur on caps that are properly stored, and the organisms present on caps are added directly.

Smith[29] found in the examination of 2,166 bottle caps, taken in lots of 28 to 68 from the supplies in use at different pasteurization plants, that if the caps had been used on bottles of milk they would have added from <1 to 453 organisms per milliliter of milk. The method of examination used was to wash a number of caps in sterile water, determine the bacteria in the water, and then calculate to one-half the surface of a cap.

As caps are handled in modern capping equipment, they are of no significance as a source of organisms in milk. However, when carelessly handled they may be of importance. Capping bottles by hand is very objectionable from the bacteriological standpoint and is prohibited by various regulations.

Paper milk bottles. Following extensive study of the paperboard used in their manufacture, methods of moisture proofing, etc., paper milk bottles now are accepted as satisfactory containers for milk. Normally, they contain very few organisms when filled and adequately protect milk against contamination; also, they are accepted by most consumers. Tanner[79] emphasized that careful methods of manufacture result in a paper container with a very low bacterial count and that pathogens cannot survive the various procedures involved.

Paperboard used for paper bottles, milk bottle caps, etc., can be examined for numbers of bacteria by disintegrating and plating.[24] Standards for the material have been established. The finished containers are examined by a rinse procedure, sterility test, etc.

Wheaton *et al.*[80] found with 4,933 paraffined containers that about 80 per cent yielded no colonies on agar plates while over 90 per cent of the remainder showed fewer than 5 colonies per container, the great majority showing but 1 colony per bottle. Mudge and Foord,[81] using the needle puncture method, noted that about 95 per cent of the paraffined half-pint containers were sterile, and a somewhat smaller percentage of the quart containers. In the trials of Rice,[82] broth sterility tests made on a total of 1,600 paper containers, selected uniformly from several moisture-proofing treatments, indicated that approximately 80 per cent were sterile. Plate counts of water rinses from 276 containers, chosen from the moisture-proofing processes, revealed approximately 60 per cent were sterile; only 10 containers yielded more than 5 colonies per container. Prucha and Tracy [83] found the paper milk containers on the market were in excellent sanitary condition. Of 2,607 quart containers, fabricated at several carton factories from paperboard manufactured at different mills, 878 (34%) were sterile and those not completely freed of bacteria contained, as a rule, only a few; tests for coliform organisms on many of the containers were negative. Bacteria did not penetrate the walls or seams of paper containers. After containers had been submerged in a bacterial suspension for as long as 48 hr, none of the bacteria from the suspension were in the milk. When containers were heavily contaminated and then paraffined, only an occasional container showed the presence of the inoculated organism. In one experiment 1,200 containers were handled in the usual way except that the operator heavily contaminated his hands by dipping them in a bacterial suspension. The containers were paraffined for about 24 sec at 76.7, 82.2, and 87.8 C (170, 180, and 190 F); all were free of the inoculated organism except one paraffined at 87.8 C.

Prucha and Tracy [83] reported that, when containers were not packed in cases, the temperature of the milk in paper changed a little more slowly than in glass; when packed in cases that were completely filled, the temperature change definitely was slower in the paper. All the paper bottles tested stood as much as 80 lb pressure before they leaked. The authors noted that paperboard commonly contains some bacteria, mostly in the spore stage, which cannot be destroyed by the heat of the paraffin. The practically sterile condition of the containers indicates that apparently the paraffin imprisons most of the organisms and keeps them from getting into the milk.

In studying the bactericidal effect of the paraffining of paperboard used for paper milk containers, Moss *et al.*[84] used a culture of *E. coli* having a thermal resistance such that a skim milk culture in sterile skim milk showed a 99 per cent reduction at 60 C (140 F) for 20 min. Under the test procedure followed, in which moisture conditions of the paperboard were controlled, 99 per cent reduction of the test organism due to paraffining was obtained in about 10 sec at 82.2 C (180 F); 20 sec at 79.4 C (175 F); 35 sec at 76.7 C (170 F); 2 min at 73.9 C (165 F); and 4 min at 71.1 C (160 F). The authors suggested that if use of relatively high paraffin temperatures is impracticable, it may be necessary to subject the containers to separate bactericidal and water-proofing treatments or provide means for increasing the bactericidal effect of the paraffining. Relatively low paraffin temperatures sometimes are desired to reduce excessive drain off before cooling which may leave fibers unprotected. Various studies suggest that, in the absence of water, the sterilizing action obtained by heating is comparable to that obtained with dry air.

Contamination from plant employees. Employees in plants handling milk and cream are a source of organisms comparable to employees on dairy farms. Because the contamination of milk from human sources may include pathogens, it is especially serious when it occurs subsequent to pasteurization, since this process so regularly is assumed to provide definite protection. Modern large-scale milk equipment, which almost entirely eliminates direct human contact with the milk after pasteurization, constitutes an important advance from the sanitary standpoint.

Contamination in homes. After delivery of milk and cream to the homes in which it is to be used there still is an opportunity for contamination. Organisms come especially from dishes and utensils and, in general, milk and cream should be left in the bottles employed for delivery until immediately before use. The practice of pouring bottled milk into a pan and allowing it to stand so that it can be skimmed easily is especially objectionable, not only from the standpoint of contamination but also because it permits rapid absorption of odors of other foods placed near the milk. Mixing milk or cream from different lots without immediate consumption often leads to souring and other changes, when these would not have occurred if one lot had been used completely before the next was opened.

In some instances one consumer out of a considerable number complains that milk sours readily or undergoes some other objectionable change. Commonly, this is due either to serious contamination in the home or to a holding temperature that is very favorable for growth of organisms.

With the types of bottle caps in common use there is an opportunity for contamination of the lip of a bottle, especially from flies, persons, and the air, and many of the organisms are carried into the milk when it is poured. Jackley[85] noted the presence of *Escherichia-Aerobacter* organisms on the tops of bottles of milk purchased on the market. Isaacs and Zeiber[86] made a comparative study of milk in bottles with single and double caps. They concluded that bacteria were much more numerous on single caps than on the inner of the double caps. Anaerobic, sugar-fermenting organisms were found on the single caps but not on the double caps; *E. coli* was more frequently present on the single caps (61 per cent compared to 13.6 per cent). With the single caps the bacterial counts on the surface cream averaged 51 to 121 per cent greater than with the double caps, and *E. coli* was present with much greater frequency. On the average the counts on the whole milk were between 38 and 64 per cent greater with the single caps. McFarlane and Weinzirl[87] studied expansion and contraction in milk bottles and noted that a bottle does not necessarily need to be full, or even to exude cream, in order to draw liquids from the surface of the cap into the cream. They also noted that small quantities of cream may exude above the surface of the cap even when the expansion on warming did not completely fill the space beneath the cap. The investigators concluded there are more contaminating organisms on the inner lips and disc caps of single-capped milk bottles than on the inner lips and disc caps of double-capped bottles.

Arnold[88] determined the influence of the dripping of milk down the side of the bottle on the contamination of subsequent samples poured from the bottle. Quart bottles with common sense finishes were sterilized and filled with fresh pasteurized milk. The external surface of the bottle was seeded with a 24-hr culture of *Ser. marcescens* and the culture fluid allowed to evaporate. Then one glass of milk was poured and discarded and two successive pours were made into sterile glasses. From each glass 5 ml of milk was removed and divided between two agar plates. With 180 bottles examined in this manner, 38 (21%) of the second and third pours were contaminated with *Ser. marcescens*, indicating the drip returned to the top of the bottle and was transferred to the glass of milk during pouring.

CHEMICAL STERILIZATION OF DAIRY EQUIPMENT

Hot water and steam are widely used for the destruction of organisms in dairy utensils and equipment, and they are very effective when properly applied. Chemical sterilization also is employed and provides a method of treatment that is applicable when hot water or steam is inadvisable or not available, and in certain cases when it is desirable to sterilize equipment rapidly just before use.

Chemicals have been extensively employed in the sterilization of milking machines. On many farms steam is not available and the supply of hot water is limited so that chemical sterilization meets a real need. Chemical treatment of milk bottles has proved useful in certain instances when a washing machine or steam chamber was not available; frequently, it is employed as a final step in the washing of bottles with modern equipment. Chemical sterilization also has been advocated for equipment that is difficult to clean and sterilize because of its complicated construction.

Chlorine compounds. In the dairy industry chlorine compounds, including chlorine gas, hypochlorites, and chloramine-T, have been used widely as sterilizing agents. The materials are actively germicidal, and the odors are not objectionable and do not influence the flavor of dairy products when used in normal amounts. In some instances a chlorine compound is combined with a washing powder to increase the destruction of organisms during the washing process.

Chlorine gas can be passed into water and used as a rinse or sodium hypochlorite can be prepared from it. Sodium and calcium hypochlorites are marketed under various trade names; calcium hypochlorite sometimes is changed to the sodium salt before use. Chloramine-T contains chlorine in a form available for germicidal purposes and is marketed under various trade designations. It generally is considered to act more slowly than hypochlorites of the same chlorine concentration which may be an advantage in certain cases and a disadvantage in others. Loveless [89] found that acidification and raising the temperature of chloramine-T solutions increased the germicidal activity but not to the degree shown by hypochlorites.

Certain chlorine preparations may deteriorate strikingly on prolonged storage and the stability often is improved by buffering with alkaline salts. Some manufacturers employ this relationship in making their products.

Unless dairy equipment that is to be treated with chemicals is thoroughly cleaned the action is seriously influenced. Prucha[90] found that chloramine-T and sodium hypochlorite lost little or none of the chlorine when exposed for 30 min at temperatures ranging from 26.7 to 71.1 C (80 to 160 F), but that addition of a small amount of milk promptly caused a reduction in the available chlorine, which became greater with an increase in the temperature or the amount of milk added. In the absence of organic matter addition of 1 per cent sodium hydroxide to sodium hypochlorite solution increased the stability, but in the presence of organic matter the available chlorine disappeared faster with the sodium hydroxide added. The investigator noted slower destruction of *E. coli* when small amounts of milk were added to solutions of various chlorine compounds than when the milk was absent.

Various investigators have studied the effect of chlorine compounds on organisms important in dairy products. Johns[91] tested three hypochlorite and two chloramine-T products against a mixed flora from milk cans and pure cultures of *E. coli, A. aerogenes, S. lactis,* and *B. subtilis.* The liquid hypochlorite preparations were effective against all the non-spore formers, whereas a dry hypochlorite preparation was effective against the gas-forming species, slower against the mixed flora, and especially slow against *S. lactis;* the slow action against *S. lactis* was considered to be due to high hydroxyl-ion concentration. The chloramine-T products acted too slowly to be considered suitable for sterilizing rinses. None of the five products had a noticeable effect on spores of *B. subtilis* during a 2-min exposure. Later, Johns[92] studied the efficiencies of three solutions of hypochlorite preparations in the destruction of five ropy and two bitter milk organisms. The less alkaline hypochlorites were extremely effective against all seven test organisms; with a more alkaline product the Gram-positive species exhibited varying degrees of resistance.

Loveless[89] noted that when the utensils on 20 Vermont farms were rinsed with hypochlorite solution twice daily immediately before milking, the average quality of the milk, as measured by plate counts and methylene blue tests, was materially improved. Milk handled in cans treated at the farm with a chlorine rinse in addition to the treatment given at the creamery showed less contamination than portions of the same lots of milk handled in unrinsed cans. Milk produced under experimental conditions where hypochlorite solutions were employed in treating utensils compared favorably in quality with that produced in steamed utensils. When rinsed with a quick-acting hypochlorite preparation, artificially contaminated milk pails had relatively few bacteria on their surfaces.

Some non-spore-forming organisms may be relatively resistant to the action of certain chlorine compounds. Wade *et al.*[93] reported that, from their experiments, it appears a calcium hypochlorite solution containing available chlorine in amounts usually advised for treating equipment and utensils in milk plants, and even up to 500 ppm, cannot be depended on to destroy human *M. tuberculosis.* Chloramine, in a strength of 93 to 95 ppm of available chlorine, for a period of 3 min was effective. Costigan[94] found that a hypochlorite solution of low alkalinity containing 50 ppm available chlorine destroyed *M. tuberculosis* in 2.5 min at 50 C (122 F), in 1 min at 55 C (131 F), and in 0.5 min at 60 C (140 F); with higher concentration of chlorine destruction was more rapid.

A number of factors influence the germicidal activity of chlorine compounds, such as *p*H, period of action, temperature, amount and character of organic matter present, etc., so that the evaluation of the different compounds is complicated. Johns[95] noted that with hypochlorites the influence of *p*H is even greater than that of concentration of available chlorine, particularly in dilute solution. Usually the chlorine solutions used in dairy utensils and equipment contain from 50 to 200 ppm.

In considering the mechanism of the action of chlorine compounds, Charlton and Levine[96] concluded that in disinfection with hypochlorites the undissociated hypochlorous acid is the most significant germicidal agent, whereas with chloramine-T this acid does not play the significant role.

The corrosive action of chlorine derivatives on metals requires consideration whenever these compounds are employed. Action of the different derivatives varies widely with the metals used in dairy equipment and is affected by various factors. Loveless[89] noted that as alkalinity increased the amount of corrosion from hypochlorite was lowered and that chloramine showed little corrosive action.

Quaternary ammonium compounds. Quaternary ammonium compounds, in which various organic groups replace the four hydrogen atoms in ammonium chloride or bromide, have been developed for use in destruction of organisms in connection with preparation and handling of various foods. In general, they have a high germicidal activity, little odor or taste in normal concentrations, high stability, are noncorrosive, and have only low toxicity. In aqueous solutions they dissociate into two ions, one being the simple chloride or bromide and the other the nitrogen-carbon structure or cation. The latter is responsible for the germicidal activity and the compounds are called cationic germicides. Since the compounds are wetting agents their water solutions have low surface tensions and they contact and kill organisms not accessible to non-wetting germicides. Also, they possess detergent properties.

Dunn[97] reported that a mixture of high molecular alkyl-dimethyl-benzyl ammonium chlorides showed high germicidal efficiency toward a series of bacteria both in the presence and absence of organic matter. Specificity of germicidal action was not demonstrated to any marked degree. The material was soluble in water and its aqueous solutions had low surface tensions. It was more rapid in its action against *B. subtilis* when the reaction was distinctly alkaline, rather than acid or neutral.

Krog and Marshall[98] found that alkyl-dimethyl-benzyl ammonium chloride exerted a definite bactericidal action on the bacterial flora associated with milk-handling equipment and that it is stable when used to sanitize properly cleaned pasteurizing equipment. Frayer[99] investigated the same compound and noted that it is a highly effective germicide which is quick acting in the higher and progressively active in the lower concentrations as the time interval is increased. Additional studies on a number of the quaternary compounds have shown their efficiencies as germicides and indicated their applications in various fields.

In investigating the effect of one of the compounds on milk, DuBois and Dibblee[100] concluded that it lacks any influence on the bacterial counts of raw or pasteurized milk over a rather wide concentration range. The higher concentrations had an inhibitory effect on growth of Gram-positive, acid-producing organisms, but not on that of Gram-negative organisms.

The quaternary ammonium compounds are affected less by organic matter than are the chlorine compounds. This is a distinct advantage. They are active in neutral or alkaline solution. This often is an advantage. Botwright[101] emphasized that sanitizing rinses often follow cleaning solutions in which alkaline detergents are used so that compounds active in the alkaline range are desirable. The quaternary compounds are not compatible with some of the cleaning materials used, such as soaps, hexametaphosphate, certain wetting agents, etc.

The range of possibilities in connection with the chemical structure of the quaternary ammonium compounds that can be prepared suggests that compounds with somewhat different properties for somewhat different purposes can be developed. Studies on the effect of constitution on the germicidal properties of the compounds already indicate progress along this general line.[102]

Action of washing powders. The washing powders used in dairy plants have a destructive action on organisms, especially when used in strong solutions and at washing temperatures. This action is a factor in the value derived from washing powders in the cleaning of equipment, and in certain bottle washers it is very important. Levine *et al.*[103] studied the germicidal action of sodium hydroxide, sodium carbonate, and trisodium phosphate on dried bacterial spores at 69 to 70 C (156.2 to 158 F) and concluded that factors other than hydrogen-ion concentration were involved. When each of the compounds studied was considered individually, germicidal efficiency was a direct function of hydrogen-ion concentration. The investigators [104] also reported results indicating that the presence of sodium carbonate enhanced the disinfecting powers of sodium hydroxide, and suggested that undissociated sodium hydroxide, rather than hydroxyl-ion concentration, may be the controlling factor in disinfection with alkali. Levine *et al.*[105] noted that addition of sodium chloride, sodium carbonate, or trisodium phosphate to sodium hydroxide markedly decreased the killing time at 50 C (122 F) and 60 C (140 F); as the concentration of salts added to the hydroxide was increased, killing time was decreased but at a decreasing rate.

Mudge and Lawler [106] studied the action of alkali solutions on the bacteria found in milk bottles returned to a plant. The organisms were obtained by shaking out dirty bottles with sterile water, which was then collected in a container; suspensions of organisms thus obtained were exposed to various concentrations of alkali. Hydrogen-ion concentration was found to be an important factor in the germicidal action, as were also time and temperature. Myers [107] reported that commercial washing powders giving solutions of high *p*H were distinctly more effective as germicides than those giving solutions of low *p*H, a powder containing chlorine being an exception. Solutions that had low *p*H values and, accordingly, weak germicidal action became more effective when the *p*H was increased by addition of sodium hydroxide. Germicidal efficiency of the washing powders was not decreased to the same degree by dilution. A powder containing chlorine showed a very slight decrease in germicidal power when diluted considerably, and the others showed varying decreases. Decreases in efficiency in these alkaline solutions appeared to be closely correlated with decreases in hydroxyl-ion concentration when considered in reference to dilutions of the same powder.

Acid cleaners. Preparations containing acid and certain other ingredients, such as wetting agents and corrosion inhibitors, have been developed for use in washing equipment, cans, etc., in dairy plants.[108] One of these materials was studied by Frayer[99] and found to exert a germicidal effect, within the range of concentrations studied, which was independent of any detergent value as well as of the temperatures recommended by the manufacturer as a necessary part of the cleaning and sterilizing process.

Wetting agents. Various wetting agents that have been suggested for different domestic and industrial washing operations also are being employed in the dairy industry. They vary widely in composition. Many of them have an inhibiting effect on organisms which may vary from one organism to another. For example, Cowles[109] concluded that sodium alkyl sulfates exert a pronounced inhibitory effect upon Gram-positive organisms but not upon Gram-negative organisms. Birkeland and Steinhaus[110] found that sodium lauryl sulfate possessed a high degree of bacteriostatic activity; growth of Gram-positive bacteria and of molds was definitely inhibited, whereas most Gram-negative bacteria grew freely in the presence of the compound.

MILK PRESERVATIVES

Milk preservatives, some of which were sold under trade names, were at one time rather widely used, but regulations prohibiting the practice have largely eliminated it. The present availability of ice and refrigerating machinery provides adequate facilities for holding milk and cream so there is no longer need to use preservatives, except in samples intended for special purposes.

General effect on organisms in milk. Action of a preservative on the organisms in milk and cream depends on the amount used. A given concentration may simply prevent growth or decrease its rate, whereas a higher concentration may destroy organisms. The period a given preservative in a certain concentration will prevent changes in milk varies a great deal and depends on the species present and the temperature. A preservative commonly acts differently on different organisms and, as a result, the proportion of the various species present in a lot of milk may be definitely modified. This is objectionable if the acid-producing organisms are prevented from growing and those attacking protein are not so effectively checked. In general, the lower the temperature of holding milk or cream containing a preservative, the longer the organisms are restrained.

Preservatives that have been used in milk. The more important preservatives that were at one time used in milk intended for consumption are formaldehyde, boric acid, borax, benzoic acid, and salicylic acid. In suitable concentrations these compounds definitely delay growth of organisms. Various carbonates also have been added to milk but their main effect is to neutralize the acid produced rather than to prevent growth of organisms.

Formaldehyde. Formaldehyde is very effective in limiting growth of organisms in milk and its action is illustrated by the work of Chester and Brown.[111] The investigators found that when the concentration of formaldehyde in milk was from 1 to 2,000 to 1 to 800 there was a rapid decrease in bacteria during the first 24 hr and then a slow decrease, until at the end of 5 days the milk was practically sterile or contained only resistant spores. With a proportion of 1 to 5,000 there was rapid reduction in the bacteria for 4 to 6 hr and then a slower decrease, after which multiplication began. A ratio of 1 to 10,000 gave, in some cases, a slight decrease before the increase started, whereas 1 to 20,000 simply delayed the increase. With a proportion of 1 to 40,000 the increase in bacteria was rapid from the first but less rapid than without formaldehyde and the milk kept two to three times as long as untreated milk. *Streptococcus lactis* appeared to develop better in the presence of formaldehyde than the other bacteria ordinarily found in milk.

Hydrogen peroxide. Use of hydrogen peroxide in combination with heat was introduced as a method of treating milk by Budde and the product was called Buddeized milk. The milk was heated to 50 C (122 F) and enough hydrogen peroxide added to give a concentration of 0.03 to 0.035 per cent. After being stirred for 15 to 30 min the milk was drawn into bottles, tightly stoppered, the bottles held in a water bath at 50 C for 2 to 3 hr, and then cooled. It appears that pathogens may not be destroyed by this method. An excess of hydrogen peroxide gives an off flavor to the milk.

Chlorine compounds. The efficiency of chlorine compounds in the sterilization of dairy equipment has led to studies on their action in milk. Most investigators have found that they destroy relatively few organisms. Zoller and Eaton[112] noted that sodium hypochlorite has a relatively low bactericidal effect in milk, considering its vigorous action on bacterial cells. Only when there was an excess of the hypochlorite present, as indicated by tests, did they obtain more than a 50 per cent reduction in plate counts, and then the highest reduction was 80.8 per cent. Prucha [113] reported that large amounts of chemical sterilizers are required to produce any effect on bacteria in milk, and that milk will acquire an off flavor and be spoiled before the bacteria in it are affected. Young [114] found that preparations containing sodium hypochlorite did not materially reduce the bacterial count on milk when used in concentrations as high as 1 to 5,000 available chlorine. An odor and taste could be detected in concentrations of 1 to 20,000 and were noticeable in concentrations of 1 to 10,000. He concluded that the unpleasant odor and taste produced when sodium hypochlorite is added to milk in sufficient quantities to act as a preservative render its use impractical. Johns [115] reported that formaldehyde was a much more effective milk preservative than two hypochlorite and two chloramine-T products studied. When added to freshly drawn milk, which was quickly cooled, in the maximum concentrations not detectable by taste after 18 hr at less than 15.6 C (60 F), the preservative effect of each of the chlorine compounds was relatively slight; if the milk was not promptly cooled the effect was more noticeable, yet the bacterial count increased so greatly as to preclude the possibility of chlorine preservatives replacing prompt and thorough cooling. Hauser and King [116] found that addition of hypochlorite to fresh milk may appreciably reduce the bacterial content whether or not there is subsequent pasteurization and that hypochlorites added to milk in amounts not readily detectable by taste may produce a mild but definite preservative effect.

Preservation of milk and cream for analytical purposes. Milk and cream intended for analytical purposes can be treated with a preservative that will not interfere with the analyses to be made. Samples for fat determinations must often be held for extended periods at temperatures favorable for growth and bichloride of mercury [117] is frequently used for their preservation because of its toxic effect on all types of micro-organisms. The chemical is available in tablets which also contain some coloring material so that the preserved milk or cream will have a very abnormal appearance and not be consumed accidentally. When a preservative is used with milk or cream samples it must be well distributed if growth of organisms is to be prevented in all parts of a sample. Molds sometimes develop on samples preserved with bichloride of mercury because the reagent is not thoroughly distributed.

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Chapter 5

Bacteriology of Filtration, Clarification, and Separation

Filtration of milk is practiced on many farms and in some dairy plants, and clarification is extensively used in market milk plants and also in plants manufacturing milk products. These processes are intended primarily to remove sediment from milk. With filtration sediment is retained on the filter medium, and with clarification sediment is thrown into the bowl along with significant amounts of other milk constituents. Separation is widely used in the dairy industry to obtain cream for various purposes or to standardize the fat content of milk for such products as market milk, evaporated milk, sweetened condensed milk, milk powder, cheeses, etc.; it also collects sediment and certain milk constituents in the bowl. The three processes involve bacteriological considerations that are more or less related.

FILTRATION

In the filtering of milk, the materials most commonly used are cotton discs, or webs, and various types of cloth, some of which are manufactured especially for this purpose. In order to prevent excessive pressure at any point, the filtering material often is supported by a wire screen or some other device. Cotton webs can be single or double faced to make them more resistant to damage during use. Special types of milk filters, intended to speed up the filtration of large volumes of milk, frequently are employed. Some have additional advantages; for example, the milk may be passed through the equipment in such a way that the sediment tends to fall away from the filtering material and is not broken up and driven through or into it by pressure of the milk. Either gravity or a pump may be used to carry milk through a filter. Gathered cream intended for the manufacture of butter often is filtered through a special filter cloth or metal screen.

General effect. The principal accomplishment of filtration of milk is the removal of a considerable portion of the visible dirt. Ordinarily, this is regarded as of sufficient importance to justify the practice. It would be ideal if all milk could be produced under such conditions that there would be no dirt in it, but even with excellent conditions some sediment is present. Because of the expense involved, special production methods are not applicable to milk going into ordinary supplies. If sediment regularly is present in milk, it seems logical to remove as much of it as possible. Removal of sediment from milk has been criticized on the basis that the purchaser is given the wrong impression of the production methods, but it is improbable that anyone familiar with the milk industry ever is misled. Filtration should not be used to conceal unsatisfactory methods of milk production, and excessive amounts of sediment in milk or cream justify its rejection, but the improvement in appearance of milk that results from removal of a reasonable amount of sediment is an accepted practice that is comparable to methods used in making other food, products more attractive in appearance.

Various factors influence the rate and efficiency of milk filtration. In addition to the type of filter and filtering material, the following properties of the milk are important: content of extraneous matter, presence of certain products of udder infections, temperature, and composition, especially fat content.

Effect on bacterial content. In general, filtration of milk has only a negligible effect on the bacterial content. The particles of material removed contain organisms, although some of those originally present are distributed through the milk. It is probable that the pouring or pumping of milk against a filter tends to break up some of the sediment and thus set free more organisms than if the particles of dirt had remained intact; this may be responsible for the slight increase in bacterial count sometimes noted with filtration.

Stocking[1] strained milk through two thicknesses of cheesecloth, supported by a wire gauze, immediately after it was drawn. In seven trials the dirt in the unstrained milk averaged 0.0593 gm per liter and in the strained milk 0.0317; the dirt removed by straining varied from 10.8 to 91.8 per cent and averaged 46.6. The average numbers of organisms per milliliter in unstrained and strained milk, respectively, were 11,400 and 10,150 total bacteria; 2,900 and 2,400 acid-producing bacteria; and 873 and 882 liquefying bacteria. In the individual trials the differences in counts before and after straining were too small to be of significance. The period required to curdle at 21.1 C (70 F) was the same with strained and unstrained milk in four comparisons; strained samples curdled more quickly in the other three. Conn and Stocking[2] made ten comparisons of the bacteria in unstrained and strained milk, using litmus lactose gelatin plates; the milk was drawn from individual cows in an ordinary pail and, after removal of a sample, was strained through two layers of sterilized cheesecloth. The average counts per milliliter on unstrained and strained milk, respectively, were 13,743 and 13,005 total bacteria; 3,523 and 2,703 acid-producing bacteria; and 679 and 617 liquefying bacteria. The period required to curdle at 21.1 C varied greatly but on the average the unstrained samples curdled 4 hr earlier than the strained.

Effect of filtering milk through three separate layers of absorbent cotton in a special apparatus used on a farm producing milk of high sanitary quality was studied by Heinemann *et al.*[3] Samples were taken from the milk in the can, from the top of the strainer, and after straining. Five sets of samples were obtained each day for 16 days. Bacterial counts were made by the plate method, using 1 per cent glucose agar. The average counts per milliliter were: before straining 2,060; on top of strainer 3,912; and after straining 2,790. The investigators suggested that the higher counts on top of the strainer and after straining may have been due to breaking up the clumps of bacteria by the agitation.

Effect of filtering milk in an apparatus in which the flow was upward through a filter cloth was studied by Lucas *et al.*[4] Bacterial counts were determined on a milk powder agar incubated at 37 C (98.6 F) for 48 hr. With 68 lots of milk filtered at 29.4 to 32.2 C (85 to 90 F), the effects on the bacterial counts were: 18 (26%) of the lots showed increases of 3 to 81 per cent (avg 24.4%), 44 (65%) showed decreases of 2 to 53 per cent (avg 19.3%), and 6 (9%) showed no change. With 74 lots filtered at 12.8 to 21.1 C (55 to 70 F), the following changes in the counts occurred: 20 (27%) of the lots showed increases of 4 to 114 per cent (avg 24.7%), 40 (54%) showed decreases of 5 to 88 per cent (avg 29.3%, and 14 (19%) showed no change. The results indicate that the filter tended to lower rather than increase the plate count but a reduction was by no means regularly obtained. The filter had very little selective action in removing bacteria, although peptonizers seemed to pass through more readily than other groups. Filtration had no appreciable effect on the keeping qualities or the cream line of milk. It was very effective in removing visible dirt, being almost as efficient as clarification.

Jacobsen and Olson[5] noted that filtering milk through a special type filter had only a slight effect on the bacterial content, as determined by either the plate method or direct count. The filtered milk showed slightly poorer keeping qualities but the decrease was not uniform. Period and temperature of holding and agitation during pumping were considered to be factors in lowering the keeping qualities as well as the filtration itself. When carried out at 15.6 C (60 F) filtration had no appreciable effect on the cream line but at 32.2 or 43.3 C (90 or 110 F) it was decreased slightly.

Contamination from filters. In considering the effect of filtration on the bacterial content of milk, the possibility of the equipment, especially a previously used filter cloth, being the source of large numbers of bacteria must be recognized. When a cloth is carelessly washed, without any attempt at sterilization, or when it is left moist for a considerable period so that growth of organisms can occur, heavy contamination of the milk during the next use is to be expected. In general, the species of organisms added are very undesirable because of their ability to grow rapidly in milk.

A filter cloth that is to be used again should be thoroughly washed, then boiled or steamed, and quickly dried if it is to be kept in a suitable condition. Such treatment tends to reduce the efficiency. Lucas *et al.*[4] found that a filter cloth used once and sterilized was still fairly efficient but, for dirty milk, the efficiency was reduced to approximately 80 per cent by a second washing; they noted that with fairly clean milk a cloth can be used successfully longer than with dirty milk. In many plants employing filters, a cloth is used only once.

In two milk plants, one using a gravity filter and one a pressure filter, Dahlberg and Marquardt[6] found that the accumulation of sediment on the filter cloth did not affect the efficiency of the filter; they pointed out that this suggests the sediment remained on the cloth and was not washed into the milk. Leucocytes were not removed by filtration.

The various materials used to filter milk ordinarily come from the manufacturer in a satisfactory condition, bacteriologically, but they may be contaminated by exposure. They also may be contaminated by careless handling; such contamination is common with the cotton discs used to filter milk on farms. Contamination of filters is especially serious when they are being used for pasteurized milk or milk to be sold raw.

CLARIFICATION

Clarification of milk had its beginning in the employment of a separator to throw out the foreign material gaining entrance during production and handling. There were certain objections to the use of a separator for this purpose, such as the necessity of mixing the skim milk and cream and occasional interference with the creaming ability of the remixed milk and, accordingly, machines were designed for removal of sediment instead of separation of the fat. Clarifiers now are widely employed in the market milk industry and also with milk intended for manufacturing purposes.

Composition of slime. The slime collecting in the bowl of a clarifier is made up of dirt of various kinds that has fallen into the milk, body cells, bacteria, particles of casein, fat, and various other materials. Erythrocytes are present occasionally, even when appearance of the milk does not suggest that it contains blood, and these may be deposited as a very definite layer in the slime.

With 8 samples of clarifier slime, McInerney [7] found the average percentage composition to be water 71.33, total solids 28.67, fat 4.0, ash 3.15, nitrogen 0.33, and casein 2.13. Jacobsen and Olson [5] reported that on a moisture-free basis the material removed by clarification averaged about 60 per cent protein, 8 per cent fat, and 10 per cent ash; with various clarification temperatures the amount of protein and fat in the slime was greatest at the lowest temperature, but the amount of ash was rather constant.

The bacterial content of clarifier slime varies a great deal, presumably because of variations in the ratio of bacteria to other slime constituents. Hammer [8] noted that 36 samples of slime contained 103,500,000 to 20,000,000,000 bacteria per gram. Hammer and Hauser [9] studied 14 samples and found 7,800,000 to 5,210,000,000 bacteria per gram; in general, slime with a high bacterial count came from milk containing many organisms. Results reported by Marshall and Hood [10] also indicate that the bacterial content of the milk influences the bacterial content of the slime; the numbers of bacteria in 35 samples of slime from certified milk, most of which represented milk from individual animals, ranged from 17,000 to 650,000 per gram, whereas the numbers in 15 samples from ordinary market milk varied from 900,000 to 750,000,000.

Numbers of body cells in clarifier slime also show great variations. Hammer [8] found 565,000,000 to 1,295,000,000 cells per gram in 36 samples of slime; Hammer and Hauser [9] noted 57,000,000 to 658,000,000 in 14 samples. With 23 samples of slime from certified milk, most of which represented milk from individual animals, Marshall and Hood [10] found the cell contents varied from 3,000,000 to 670,000,000 per gram; with 6 samples from ordinary market milk the numbers ranged from 200,000,000 to 420,000,000.

Amount of slime. The amount of clarifier slime obtained from milk is extremely variable and depends, to a large extent, on the amount of dirt and the number of body cells in the milk. Other factors sometimes are involved; for example, development of a small amount of acid in milk may so influence the casein that considerable quantities are thrown into the slime. In all cases, however, the amount of slime is small compared to the volume of milk clarified. McInerney[7] found in eight trials, each involving several thousand pounds of milk, that the weight of slime varied from 0.0063 to 0.0149 per cent of the weight of milk. Marshall and Hood[10] reported the work of Davies on clarification of small lots of certified milk from individual cows. The slime obtained in 440 trials on milk from 74 animals averaged 0.067 per cent. The cows varied greatly in amount of slime in the milk under apparently normal conditions, and with individual animals there often was a variation from one milking to the next. The amount of slime was increased by abnormalities of the udder or teats. Cows that had just freshened gave a high percentage of slime which often continued for several weeks. Jacobsen and Olson[5] reported the dry weights of slime removed per 1,000 lb of milk were 39.08 gm in eight trials at 15.6 C (60 F), 28.02 gm in ten trials at 32.2 C (90 F), and 24.14 gm in six trials at 43.3 C (110 F).

Effect on bacterial content. The bacterial content of clarifier slime shows that organisms regularly are removed from milk by clarification. However, the number of bacteria, as determined by the plate method, often is greater in clarified milk than in unclarified milk from the same lot. The increase is due to breaking up of clumps of bacteria during the agitation in the machine so that a clump that would have given only one colony before clarification gives several colonies on the plates poured from the clarified milk; accordingly, the increase is an apparent one. Removal of bacteria and breaking up of clumps are the two factors tending to change the number of bacteria during clarification, and whether there will be a decrease or an apparent increase is determined by which of these factors has the greater influence.

Hammer,[8] using the plate method, compared the bacterial content of milk before and after clarification. In 51 comparisons on milk containing less than 100,000 bacteria per milliliter originally, 3 (6%) gave the same count before and after clarification, 14 (27%) showed a decrease of 2 to 24 per cent (avg 12%) after clarification, and 34 (67%) gave an increase of 2 to 256 per cent (avg 41%). In 27 comparisons on milk having between 100,000 and 500,000 bacteria per milliliter originally, 9 (33%) showed a decrease of 2 to 36 per cent (avg 12%) after clarification, and 18 (67%) showed an increase of 3 to 187 per cent (avg 43%). In 14 comparisons on milk having more than 500,000 bacteria per milliliter originally, 3 (21%) showed a decrease of 5 to 40 per cent (avg 24%) after clarification, and 11 (79%) showed an increase of 4 to 102 per cent (avg 29%).

Hammer and Hauser [9] also studied the effect of clarification on the plate count of milk. Of 52 lots of milk containing less than 100,000 bacteria per milliliter originally, 1 (2%) gave the same count before and after clarification, 8 (15%) showed a decrease of 2 to 43 per cent (avg 16%) after clarification, and 43 (83%) showed an increase of 1 to 767 per cent (avg 85%). For the 52 comparisons there was an average increase in the bacterial content of 68 per cent. In 9 comparisons on milk containing 100,000 to 500,000 bacteria per milliliter originally, 7 (78%) showed a decrease of 9 to 55 per cent (avg 19%) after clarification, and 2 (22%) showed an increase of 27 to 44 per cent (avg 35%). Considering the 9 comparisons, there was an average decrease in the bacterial content of 7 per cent. In 32 comparisons on milk containing more than 500,000 bacteria per milliliter originally, 17 (53%) showed a decrease of 1 to 31 per cent (avg 14%) after clarification, and 15 (47%) gave an increase of 3 to 50 per cent (avg 13%). If the 32 comparisons are considered, there was an average decrease in the bacterial content of 1 per cent.

McInerney,[7] using the plate method, found with 28 samples of fresh milk that in 1 clarification caused no change, in 1 it caused a decrease, and in 26 it caused an increase of 1.76 to 415.10 per cent (avg 87.15%); with 17 samples of old, dirty milk clarification increased the count in all cases, the increase varying from 5.82 to 1,574.70 per cent (avg 114.77%).

With 24 samples of milk low in bacteria, Sherman [11] found that clarification caused an increase in the plate counts in all cases; the average counts per milliliter were 4,720 before clarification and 7,120 after. Marshall and Hood [10] noted both increases and decreases in bacterial counts as a result of clarifying 37 lots of certified milk, largely from individual animals; the maximum increase was 614 per cent and the maximum decrease was 75 per cent.

Lucas *et al.*[4] studied the effect of clarification on the bacterial contents of 49 samples of milk, using temperatures ranging from 29.4 to 33.3 C (85 to 92 F); after clarification 35 (71%) showed increases which averaged 28.1 per cent (from 1 to 100%), 9 (19%) showed decreases which averaged 11.7 per cent (from 2 to 23%), and 5 (10%) showed no change. Clarification at 12.8 to 18.3 C (55 to 65 F) influenced the bacterial content of 97 samples as follows: With 60 (62%) there were increases which averaged 25.9 per cent (from 2 to 220%), with 23 (24%) there were decreases which averaged 12.2 per cent (from 3 to 26%), and with 14 (14%) there was no change.

Both increases and decreases in the bacterial content of milk as a result of clarification were noted by Wilster; [12] considering the 43 comparisons, however, there was an average increase of 12.1 per cent. When the rate of inflow of milk was reduced, clarification always caused a decrease in the plate count, the average reduction for 19 trials being 55.3 per cent. With either a normal or reduced rate of inflow, clarification had no significant effect on the types of bacteria present, or on the fermentation that occurred at a temperature favorable for bacterial growth.

Jacobsen and Olson [5] found that clarification at all the temperatures studied increased the plate count on milk. The increases averaged 134.5 per cent at 15.6 C (60 F), 36.1 per cent at 32.2 C (90 F), and 20.0 per cent at 43.3 C (110 F). Microscopic examinations indicated the increases were due to breaking up of clumps.

Weinreich [13] reported on work done by Parfitt in which considerable bacterial reductions were obtained when milk was clarified warm, at about 60 C (140 F), but increases in counts were obtained when the milk was clarified cold, at about 18.9 C (66 F). An average count of about 2,000,000 per milliliter was reduced roughly to about 200,000 (average decrease about 88%) at 60 C, while the same milk showed an increase between 3 and 15 per cent when clarified cold.

Effect on body cell content. Because body cells in milk are counted directly and there is no opportunity for apparent changes in number, the presence of body cells in clarifier slime indicates that clarified milk has a lower cell count than the same milk before clarification. Hammer [8] found with 52 lots of milk that clarification caused decreases in cell content of 7 to 73 per cent (avg 39%). In 63 lots of milk Hammer and Hauser [9] observed decreases of 11 to 78 per cent (avg 41%). In neither series of trials was there an apparent relationship between percentage of cells removed and such factors as original cell content, percentage of fat in the milk, or temperature of clarification. Marshall and Hood [10] found, with 25 samples of certified milk coming largely from individual animals, that clarification reduced the cell content from 46 to 92 per cent, whereas with 6 samples of ordinary market milk the cell content was reduced from 74 to 92 per cent. Dahlberg and Marquardt [6] reported that clarification reduced the cell content of milk 67.3 per cent. Jacobsen and Olson [5] noted that the temperature of clarification influenced the percentages of cells removed; at 15.6 C (60 F) 34.98 per cent were removed, at 32.2 C (90 F) 46.28 per cent, and at 43.3 C (110 F) 53.20 per cent.

Hahn and Tracy[14] found that single clarification, using either a clarifier unit, or a separator unit and remixing the milk, reduced the cell content on an average of 61.7 and 51 per cent, respectively. The data indicated variations in the numbers of cells removed from milk throughout the entire clarifying period. Efficiency of cell removal increased with an increase in temperature. Clarifying two or more times increased the extent of cell removal over single clarification. Pumping milk at slow speeds through an air-tight clarifier resulted in greater clarifier efficiency than pumping at higher speeds. Clarification after homogenization was more efficient than before homogenization.

Removal of visible dirt. Clarification very efficiently removes the visible dirt from milk. McInerney[7] found about 99 per cent of the dirt taken out, whereas Lucas *et al.*[4] found more than 99 per cent removed. Some of the dirt which gets into milk either goes into solution or is so finely divided it ordinarily is not visible and, accordingly, a portion of the dirt may not be removed by clarification; different types of dirt vary greatly in this respect.

Effect on keeping quality of milk. Clarification has little, if any, effect on the keeping quality of milk. Hammer and Hauser,[9] using room temperature, compared the acid development in 26 samples before and after clarification. Titrations were made following a certain amount of curdling, and in a few instances coagulation was complete. In 3 comparisons (12%) the acidity was the same, in 13 (50%) the clarified milk showed the higher acidity, and in 10 (38%) the unclarified milk showed the higher acidity. McInerney[7] compared development of bacteria in milk before and after clarification by holding samples at 14.4 C (57.9 F) for 24 hr and then plating on lactose agar and incubating the plates 3 days at 37 C (98.6 F). With clean milk the unclarified samples showed a greater percentage increase than the clarified in 9 of the 15 comparisons. The average increase for the 15 trials was 1,374.50 per cent with the unclarified milk and 754.29 per cent with the clarified. With dirty milk the unclarified samples showed a greater percentage increase than the clarified in 9 of 20 comparisons. The average increase for the unclarified milk was 1,691.86 per cent and for the clarified 431.50 per cent. McInerney also made 17 comparisons of the acidities of unclarified and clarified milk after holding 24 hr at 21.1 C (70 F) and found practically no difference, the average acidity for the unclarified milk being 0.57 per cent and for the clarified milk 0.60 per cent.

In trials with milk having a low bacterial count, Sherman[11] found that clarified milk reduced methylene blue and developed acid somewhat more rapidly than unclarified. Lucas *et al.*[4] also concluded that clarification slightly reduces the keeping quality of milk and attributed this to stimulation of the acid-producing bacteria. Wilster[12] noted in nine trials that clarification under normal or reduced inflow caused an increase in the period required for methylene blue reduction; the increase was greater with reduced than with normal inflow.

Effect of full clarifier bowl. Removal of bacteria, body cells, and sediment by clarification can only take place when there is room for them in the bowl. Weinreich[13] illustrated, with the following data, the failure of a clarifier to remove bacteria when the bowl was full:

FAILURE OF A CLARIFIER TO REMOVE BACTERIA WHEN THE BOWL IS FULL BECAUSE OF EXTENDED OPERATION

	Bacteria per Milliliter		
Time	*Raw Milk*	*Before Clarification*	*After Clarification*
7:15 A.M.	150,000	190,000	24,000
8:30	150,000	190,000	36,000
9:15	160,000	230,000	79,000
10:15	230,000	285,000	74,000
11:15	170,000	270,000	240,000
12:15 P.M.	160,000	150,000	155,000

Effect on pathogens. There is no evidence that clarification makes milk safe by removal of pathogens. Traum and Hart[15] inoculated 14 samples of clarified milk from a tuberculous herd into guinea pigs; 11 samples caused tuberculosis in all the animals inoculated, 2 produced tuberculosis in one guinea pig while the other died soon after inoculation, and 1 caused death of both guinea pigs through septicemia.

Value. The primary value of clarification lies in the removal of dirt from milk and there is the same justification for removing dirt by clarification that there is for removing it by filtering. Another advantage is the elimination of a portion of the body cells from milk; these cells cannot be considered harmful, but their removal is advisable from the aesthetic standpoint. Although clarifier slime contains large numbers of bacteria per gram, the amount of slime compared to the volume of milk is so small that clarification is of no importance in reducing the bacterial content. The effect of clarification on the keeping quality of milk also is negligible.

SEPARATION

With centrifugal separation, milk is divided into three fractions, skim milk, cream, and slime. The slime that collects in the separator bowl contains essentially the same materials as clarifier slime; as with clarification the amount is small compared to the volume of milk separated. With gravity separation the fat comes to the surface and, when it is skimmed off, the milk is divided into two fractions, skim milk and cream, no organisms being eliminated through centrifugal force. Also, with gravity separation, organisms may multiply during the period creaming is taking place. The effect of separation on the bacteria in milk is important from various aspects, such as comparative bacterial standards for milk and cream and the bacteriology of products made from cream.

Distribution of bacteria during separation. Cream frequently contains somewhat larger numbers of bacteria per milliliter than the whole milk and skim milk somewhat smaller numbers because a part of the bacteria may become entangled in the fat globule clusters and be carried to the fat-rich portion. However, some analyses, particularly of separator cream, show smaller numbers of bacteria per milliliter of cream than of skim milk and, it appears, various factors influence this distribution. Breaking up of clumps of bacteria, which is a factor in the high plate counts often obtained following clarification, also is a factor in centrifugal separation.

Eckles and Barnes [16] studied the effect of separating milk on the distribution of the bacteria present. In a typical trial the whole milk contained 58,050 bacteria per milliliter, the skim milk 16,200, the cream 98,000, and the slime approximately 38,000,000. Average counts obtained by Anderson [17] in seven trials, using centrifugal separation, were: whole milk 619,800 per milliliter, skim milk 119,700, and cream 4,500,000; in six trials with gravity separation the counts were whole milk 2,708,000, skim milk 2,050,000 and cream 15,416,000. Lamson [18] noted that there usually were less, or only slightly more, bacteria per milliliter in separator cream than in the whole milk from which it came, and the skim milk usually contained fewer bacteria than the whole milk.

Leete [19] concluded that centrifugal separation with a clean separator will not result in cream having a greatly higher count than the whole milk from which it is obtained, whereas gravity cream will give a much higher count than the whole milk. In 100 runs with a steam-turbine separator the average bacterial counts per milliliter were: whole milk 435,240, skim milk 312,740, and cream 500,830. Seventy samples of the cream showed increases in count over the corresponding whole milk with an average of 23.87 per cent; 23 samples showed decreases with an average of 14.06 per cent; with 7 samples the counts on the cream and whole milk were the same. Considering the 100 samples, Leete found the average increase of the cream over the whole milk was 14.84 per cent. The fat content of the whole milk averaged 3.905 per cent and of the cream 34.64 per cent. Both the greatest increases and the greatest decreases in the bacterial content of the cream over the whole milk occurred with low-count milk. The gravity separation was carried out in a can 18 in. high and 8.5 in. in diameter, which was carefully washed and steamed before use. During the separation the can was kept in a tank of ice and water. In 25 runs the average bacterial counts per milliliter were: whole milk 135,880, skim milk 33,556, and cream 283,680. The bacterial content of the cream was higher than that of the corresponding whole milk in every case, the average increase being 160.86 per cent, while the skim milk was lower in bacteria than the whole milk in 23 of the comparisons. The average fat content of the whole milk was 4.208 per cent and of the cream 20.52 per cent.

In the seven trials reported by Ulvin and Cree,[20] the counts on the cream were relatively low; with the raw whole milk plate count as 100 per cent, the raw skim milk averaged 125.5 per cent as many colonies and the raw cream 65.6 per cent as many as the raw whole milk. The fat content of the raw whole milk varied from about 3.9 to 4.3 per cent and that of the raw cream was approximately 25 per cent; separation temperature was 32.2 C (90 F).

Separator slime. The amount of dirt in separator slime depends largely on the amount in the original milk. In general, a separator is efficient in removing foreign matter from milk. Large numbers of body cells are present in slime; Dunn *et al.*[21] found from 700 to 900 million per gram.

Distribution of bacteria in bottled milk. Distribution of the bacteria in bottled milk was studied by Torrey and Rahe,[22] who found that the upper 2 oz of the cream of fresh bottled milk of fair quality contained, on the average, 50 to 100 per cent more bacteria than an equal amount of the lower cream, whereas with older and more grossly contaminated milk the lower cream might contain as many as or even more bacteria than the upper layers; a higher percentage of bacteria was brought to the surface layers in milk rich in fat than in milk poor in fat. After being held 1 hr in bottles the milk showed no distinct cream layer and only a slight increase in bacteria at the surface; it was not until a cream layer began to form that an excess of bacteria in the upper layers was noted.

Stine[23] found that when quart bottles of milk were held at 0 C (32 F) for 6 or 30 hr the cream had much higher bacterial counts than the milk beneath. The counts on the top of a cream layer were higher than those on the lower portions, and the counts appeared to decrease progressively at greater depths in a bottle. Added yeasts showed a greater tendency to accumulate at the top of a bottle of milk than did bacteria.

Nicholas *et al.*[24] bottled and capped fresh milk in 12 clean quart bottles, with precautions against contamination, and suspended them, in a wire crate, in the water bath of an electric milk cooler. The refrigerant came nearly to the tops of the bottles and cooling was accelerated by agitation of the water bath. At 15-min intervals 40 ml of material was removed from the top of a bottle for plating and fat determination, and the temperature of the milk was recorded. The cooling water remained between 5.6 and 6.1 C (42 and 43 F) and the milk dropped rapidly to below 10 C (50 F). Under the conditions the increase in fat at the top of a bottle was soon significant. Bacterial filtration accompanied the fat separation, resulting in a concentration of organisms in the cream layer that finally reached 97 to 99 per cent of the total count. Rate of separation and filtration was maximum between 1 and 2 hr and was practically complete in 2 hr.

Effect on pathogens. Separation does not have a selective action on the organisms in milk and there is no complete concentration of the pathogens in either the slime or cream. Moore[25] studied the effect of centrifugal separation on *M. tuberculosis* in milk and found that, although many of the organisms were thrown into the slime, both the skim milk and cream contained them.

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Chapter 6

Growth of Bacteria in Milk and Cream

The numbers of bacteria that gain entrance to milk often are increased, and in some instances extensively increased, through multiplication during the holding. The excessive counts on many lots of milk reaching dairy plants or consumers are due largely to growth of organisms, since they are much greater than the counts obtained on freshly drawn milk, even when it is produced under very insanitary conditions.

The rate at which organisms increase in milk is determined primarily by two factors, temperature of holding and species present. The holding temperature also greatly influences the types of organisms that grow, since the maximum, optimum, and minimum growth temperatures vary widely with different species and a temperature that is satisfactory for growth of one may entirely prevent development of another; even within a species there may be variations in growth rates of different strains at a given temperature.

Germicidal action of milk. For a certain period after milk is drawn there may be a decrease in the number of bacteria in it, as determined by the plate method, and this ordinarily is considered to be due to a germicidal property. Blood and body fluids in general have the ability to destroy many species of organisms and some such action would be expected with milk. The germicidal action of milk, however, is rather weak and perhaps should be looked upon as a restraining or antiseptic action rather than one actually destroying organisms, although with certain species it may be very effective. It was first detected by Fokker[1] in an investigation of goats' milk. With inoculated raw milk he observed slower souring and a smaller number of colonies on plates than with heated milk similarly inoculated. Various investigators have found that, when pure cultures of bacteria are added to raw milk and the numbers determined after varying periods, very definite decreases sometimes are obtained. Heinemann and Glenn[2] noted that, with this procedure, inoculation of fairly large numbers of bacteria into cows' milk gave a more decided decrease than inoculation of only small numbers.

The decrease in the plate count on a lot of milk for a period after production is not pronounced and clear cut and it is only through many trials by various investigators that such a decrease has been definitely established. The germicidal action of milk is influenced to a large extent by the temperature of holding. At a comparatively high temperature, such as 37 C (98.6 F), the decrease in count is complete in a few hours and a rapid increase then follows; at lower temperatures a definite increase takes considerably longer. At 0 C (32 F) there may be a gradual decrease extending over more than 1 week and it is at this temperature that a decrease in the plate count on milk during holding is the most definite.

Early in the investigations on the germicidal property of milk Hunziker [3] pointed out: "The degree of the germicidal action varies greatly in milk of different animals and sometimes in milk of the same animal taken at different milkings." Variation in the milk of individual animals also was noted by Chambers,[4] who reported that in a group of 11 cows the milk of only 3 possessed germicidal action. Jones [5] observed that the inhibitory substance varies in the milk from different quarters of the same cow and that it is present in the milk of the first days of lactation as well as later. Hanssen [6] found the property greatest in summer.

Various bacteria are influenced very differently by the germicidal action of milk and, accordingly, this property must be considered as more or less specific. The extreme variation is illustrated by samples of milk which show pronounced germicidal action for one species and none for another. Organisms which grow rapidly in milk appear to be included among the species influenced by the germicidal property. Heinemann and Glenn [2] found that *S. lactis* increases in milk from the beginning, although it may be inhibited to some extent, and Sherman and Curran [7] noted a slight but definite inhibitory effect on this organism. Chambers,[4] however, reported that the milk of two cows, which was known to possess germicidal properties, had no action on *S. lactis*. Curran [8] found that an organism capable of rapidly sweet curdling milk was markedly restrained in certain samples of mixed raw milk and coagulation was delayed from 3.5 to 6 hr in the different samples. *S. lactis* was restrained only slightly by the milk. The marked germicidal activity was present in the milk over a period of several months and was confined to Jersey milk.

Jones [9] noted that addition of milk filtered through a Berkefeld candle V, or heated at 58 C (136.4 F) for 20 min, to blood agar plates interfered with development of colonies of the scarlet fever *Strepto-*

coccus, the inhibition being proportional to the amount of milk used. When the organism was added to the milk that had been heated and the incubation prolonged, it was killed. Boiling the milk destroyed the inhibitory substance.

Action of heat on germicidal property. High temperatures destroy the germicidal property of milk but the temperature required for such destruction appears to vary with different organisms. Rosenau and McCoy[10] found that the germicidal property for *A. aerogenes* was decreased at 55 C (131 F) for 30 min and was almost destroyed at 60 C (140 F) for 20 min, whereas the action against *Eberthella typhosa* was not influenced at 60 C for 20 min but was materially decreased at 70 C (158 F) for 30 min. Freezing temperatures for short periods had no influence on the germicidal property, but freezing for long periods decreased it. Hanssen,[6] using *E. typhosa* and paratyphoid B, found that 75 C (167 F) for 15 min destroyed the bactericidal property, whereas 70 C for 15 min or 63 C (145.4 F) for 30 min did not. The earlier work of Hunziker,[3] which involved only the organisms gaining entrance to milk during production, showed that heating to 65 C (149 F) for 40 min destroyed the germicidal property. Jones and Little[11] found that the substance in milk which inhibited a mastitis *Streptococcus* was relatively resistant to heat; with 20 min exposures, 56 to 62 C (132.8 to 143.6 F) did not affect it, 65 to 70 C impaired it somewhat, and 80 C (176 F) was as destructive as boiling for 5 min.

In studies on a bactericidal substance in milk that was specific for certain strains of coliform organisms, Morris[12] found the following effect of heat:

EFFECT OF HEAT ON A BACTERICIDAL SUBSTANCE IN MILK THAT WAS SPECIFIC FOR CERTAIN COLIFORM STRAINS

Milk Heated 30 Min at		Plate Count per Milliliter *At Inoculation*	Plate Count per Milliliter *After 4 hr at 37 C (98.6 F)*
Culture 1	52 C (125.6 F)	1,328,000	1,000
	53 C (127.4 F)	848,000	40,000,000
Culture 2	52 C	316,000	31,000
	53 C	640,000	28,000,000

Causes of decreases in bacteria in milk. The action of milk in causing a decrease in the number of bacteria, as determined by the plate method, appears to be dependent on several factors. Certain bacteria apparently find conditions unfavorable and rapidly die. Some of the leucocytes present may retain their ability to ingest bacteria after the milk is drawn, although there seems to be no good evidence of this; examination of milk sediments frequently shows leucocytes containing bacteria, but these may have been ingested before the milk left the udder. Certain of the bacteria gaining entrance to milk apparently are clumped by agglutinins and the plate count thus could be decreased since, if the organisms remain clumped, they would produce fewer colonies than if agglutination had not occurred. The agglutinins undoubtedly come from the blood of the producing animal, and since variations occur in the agglutinin content of blood similar variations are to be expected in milk; this may be a factor in explaining variations in the germicidal property of different lots of milk. Clumps of organisms, some of which contain enormous numbers, are common in milk sediments and, although some of these undoubtedly are due to the manner of growth of the particular species, others may be due to agglutination. Chambers [4] found clumping of certain organisms added to milk but no direct relationship between agglutination and germicidal action. Antibodies other than agglutinins also are present in milk and these may be a factor in the germicidal property of the product.

Hanssen [6] proposed the theory that the bactericidal property of milk is in close relation to the oxidizing enzymes and suggested that variations in bactericidal power at different seasons depend on variations in the feed of the cow through their influence on the content of oxidizing enzymes in the milk.

In testing the germicidal activity of fresh raw milk with a mastitis *Streptococcus,* Jones and Little [11] found that milk of all the cows inhibited the organism although the period of inhibition varied; milk of some cows prevented growth for 8 hr, that of others for 4 or 6 hr. Action of agglutinins was not the cause of the inhibition and the inhibitory principle was not increased by immunizing the cows with the *Streptococcus.* Whey obtained from milk with rennet was about as inhibitory as the original milk. Jones and Simms [13] concluded that the mastitis *Streptococcus* becomes adapted to the inhibitory agent in milk and that sudden growth of the organism is due to this adaptation rather than to the presence of a resistant type of organism in the original culture. Later, these investigators [14] named the bacterial growth inhibitor of milk *lactenin* and studied it in some detail. Lactenic activity of whole milk was not decreased by removal of fat or coagulation with rennet. The inhibitor was stable for 1.5 hr at *p*H 4 or 10 and for longer periods in neutral solutions. It was removed by reagents which precipitated the proteins of whey. Part of these proteins could be hydrolyzed by tryptic digestion and the resulting split products, together with salts and sugar, removed by dialysis without appreciable loss of lactenic activity. When the material was dialyzed in a concentrating dialyzer and then completely desiccated, it kept for 3 mo with practically no loss of activity. The dry preparation was 200 times as active as the original milk when tested with the scarlet fever *Streptococcus.*

Curran[15] studied the comparative bacterial growth in udders of living cows and in udders following death and removal of the blood. Some bacterial species which were present in udders of living cows at a fairly constant level multiplied actively immediately following death of the cows. Rapid-growing, invasive types of streptococci were most affected. In some cases the udder flora did not increase appreciably during post-mortem incubation and then relatively inert, slow-growing micrococci and streptococci usually were involved. The suddenly increased multiplication of the udder flora that frequently attended withdrawal of the blood was interpreted as suggesting that the circulatory system is directly or indirectly associated with formation of the germicidal substance in milk.

In studies on the germicidal property of milk, the effect of various factors, including associative action, on the growth of organisms should be recognized. It appears that in some instances effects attributed to the germicidal property of milk may have been due to other causes.

Significance of germicidal property. Although the germicidal property of milk inhibits growth of certain organisms, it is of little practical importance in the holding of milk and cream, except for short periods, and cannot be expected to prevent action of organisms in these materials. However, it may have a significance not yet completely appreciated. It may be a factor in restraining growth of mastitis organisms in the udder, it may protect suckling animals against infections, and it may delay action of organisms in cheesemaking.

Little[16] reported that in the milk of all cows examined there was present a substance which inhibited growth of mastitis streptococci. Milk of young cows early in their first lactation contained about the same amount of the substance as milk from older cows. Concentration of the inhibitory principle varied in the secretions of different animals and, occasionally, in quarters of the same udder. There was some evidence that the inhibitory substance in milk was more potent against its own homologous organisms than against similar foreign varieties.

Influence of temperature on fermentation in milk. Although the germicidal property of raw milk delays bacterial growth in it, such growth eventually occurs. The temperature at which milk is held influences both the rate of bacterial growth and the species developing. Accordingly, the fermentation that occurs in raw milk containing a variety of bacteria is determined largely by the holding temperature. *S. lactis* tends to dominate the fermentation over a rather wide temperature range but it fails to grow at both lower and higher temperatures and other species then become conspicuous. The temperature at which a particular fermentation is noted under practical conditions may not be the most favorable for the causative organism but, because of the restraining action on competing species, it is comparatively very satisfactory; for example, *Alc. viscosus* grows more rapidly at 21 C (69.8 F) than at 10 C (50 F) but in milk at the former temperature *S. lactis* is so active that *Alc. viscosus* rarely produces ropiness, whereas at the latter temperature it finds much less competition.

Frozen milk and cream. In frozen milk or cream, there is no multiplication of bacteria. The methods by which bacteria obtain their nutrients preclude growth as long as the milk or cream is frozen throughout. Usually, there is a decrease in numbers, the rate being dependent on the resistance of the contained organisms to the unfavorable conditions. In milk at −9 C (15.8 F), Ravenel *et al.*[17] noted a tendency to a decrease in the plate count. Eventually, the milk developed an abnormal flavor.

Hilliard and Davis[18] concluded that, with freezing temperatures, there is much less destruction of bacteria in milk or cream than in pure tap water, owing no doubt to physical protection of the bacteria by the colloidal and solid matter in suspension. The degree of cold below freezing was not important in the destruction of bacteria but crystallization, probably resulting in mechanical crushing, appeared to be. The relationship of mechanical protection from sheering ice crystals given by solutes to viability of bacteria at low temperatures also was pointed out by Sedgwick *et al.*[19]

Weiser and Osterud[20] studied the death of bacteria at low temperatures, using *E. coli* and a suspending medium of 1 per cent peptone or a peptone buffer mixture at *p*H 7.0. They concluded that death by freezing involves (1) rapid-acting or immediate death caused by freezing and thawing, and (2) storage death which is a direct function of time and temperature. Mortality due to immediate death by freezing was marked but did not vary with intensity of the temperature; death occurred at a brief stage in the freezing process during which extracellular ice formation was being completed. Storage death at the higher freezing temperatures was very rapid and was much greater above

−30 C (−22 F) than at −30 C, or below; at −195 C (−319 F) storage death either did not take place or was not detected in the period (10 hr) studied. Repeated freezing was more lethal than single freezing or storage in the frozen state for a similar period. Freezing was much more lethal than supercooling.

Milk that has been frozen for some time does not regain its normal consistency on being thawed. Webb and Hall [21] found that slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion.

Freezing of pasteurized cream of relatively high fat content provides a convenient means of storing milk fat in periods of surplus. Value of the product is such that the storage expense often is justified. Frozen cream has been used largely in ice cream but also in cream cheese and other products. Excess supplies for which no more profitable market could be found have been churned. Physical changes during freezing may present some difficulties in its use in ice cream and perhaps other products but ordinarily homogenization or comparable procedure largely overcomes these.[22] Trout and Scheid [23] reported that the maximum destabilizing effect in the freezing and storage of cream occurred within the first 3 mo. Bell and Sanders [24] noted that differences in milk, especially in size and number of fat globules, and changes in the temperature of the milk and cream greatly influence stability of the cream emulsion, as measured by the amount of oiling off after freezing and brief storage. Later,[25] they concluded that the following precautions give frozen cream which will have a stable emulsion and good body when thawed after storage for several months: (1) use of freshly drawn milk which contains small, unclumped fat globules, (2) maintenance of these globules in a fluid, completely dispersed state until freezing, (3) quick freezing to maintain this dispersion, and (4) storage temperatures sufficiently low to prevent physical changes which cause localized crowding, distortion, and disruption of the fat globules and consequent oiling off on thawing.

Fabian and Trout [26] found that the plate count of bacteria in cream decreased during storage at −20.6 to −23.3 C (−5 to −10 F). With raw cream the averages of 12 monthly samples were 2,498,000 per ml for the fresh cream; 1,638,000 after 6 mo; and 680,000 after 12 mo. Corresponding values for cream pasteurized at 65.6 C (150 F) for 30 min were 6,795; 6,681; and 5,458, respectively. They concluded that from a bacteriological standpoint there is no reason why clean, wholesome, fresh cream cannot be pasteurized and stored for 1 yr in glass, paper, or tin containers at −20.6 to −23.3 C.

The defrosting of cream presents a definite bacteriological problem. With slow defrosting there are possibilities of bacterial growth and these should be recognized and precautions taken against them by controlling the temperature and period of holding of the melted product.

The off flavors that develop in frozen cream are chemical in nature, an oxidized flavor being the most common. Various factors influence its development. Dahle [22] concluded that pasteurization of the cream at temperatures above 76.7 C (170 F), low copper content, low storage temperatures [−17.8 to −26.1 C (0 to −15 F)] in proper containers, and homogenization at the heating temperature all aid in enhancing keeping quality. According to Trout and Scheid,[27] fresh high-quality cream pasteurized at 73.9 C (165 F) for 15 min, or at 85 C (185 F) for 5 min, withstood storage at −23.3 C (−10 F) for 3, 6, or 12 mo without appreciable development of oxidized flavor; 85 C for 5 min resulted in slightly superior keeping quality, particularly after 24 mo. With 1 ppm copper added to the cream after pasteurization, the cream invariably became oxidized, even after 3 mo, regardless of the temperature of pasteurization.

Roadhouse and Henderson [28] used a quick freezing procedure on milk and cream with the cans sealed under vacuum. Properly pasteurized milk, when frozen and stored at −20.6 C (−5 F), retained a very good flavor and body for 6 weeks; with pasteurized cream the flavor was not quite equal to fresh cream but, for 6 weeks, was considered satisfactory for use where fresh products are not available. With either product there was no advantage in homogenization with the quick freezing. Bacterial counts remained low during the 6-mo storage period.

Doan and Leeder [29] demonstrated the possibility of manufacturing frozen concentrated milk to be distributed to the ultimate consumer in much the same manner as frozen foods; the product is defrosted and reconstituted to the fluid state by thawing in hot water. The manufacturing process involves pasteurizing fresh milk at a high temperature, for example 82.2 C (180 F) for 15 min, condensing it (about 3 to 1) in the absence of copper contamination, homogenizing it efficiently, freezing it initially in an ice cream freezer (preferably a continuous type), packaging, and freezing finally in a room not above −23.3 C (−10 F). During frozen storage the bacteria remained dormant and some were destroyed; after 10 weeks the count was almost invariably lower than immediately after freezing. Thickening, and later flocculation, was an aging characteristic of the protein of the product.

During World War II frozen homogenized milk was developed to supply fresh milk to patients on hospital ships. Babcock *et al.*[30] found that homogenized milk frozen and stored at −10 C (14 F) had a slightly flat flavor when thawed after 21 days; after 89 days the flavor was definitely flat and slight separation had occurred. When frozen and stored at −32.8 C (−27 F), homogenized milk remained normal 115 days and there were indications it would remain normal for a longer period. There also were indications that with freezing and storage at still lower temperatures, for example −40 C (−40 F), the milk would remain normal longer than when frozen and stored at −32.8 C. Homogenized milk frozen and stored at one temperature was moved to a lower temperature without causing abnormalities to develop but when moved to a higher temperature it became abnormal in appearance and flavor. Trout[31] had previously reported that, upon thawing, frozen homogenized milk did not exhibit the flakiness which was common when unhomogenized milk was frozen and thawed but did exhibit a watery appearance at the surface layers which was more pronounced when the frozen milk was thawed slowly.

Preparation and transportation of frozen homogenized milk is relatively expensive, compared to preparation and transportation of milk in other forms, but its palatability may justify the expense under certain conditions.

Milk and cream at 0 C (32 F). Milk and cream sometimes are stored at approximately 0 C, which is slightly above the freezing points, and such storage constitutes a satisfactory method of preservation. Commonly, there is a decrease in the plate count for 1 week, or more. Multiplication of bacteria then begins and continues until enormous numbers are present so that the holding at 0 C should not be excessive. In milk held slightly above the freezing point, Pennington[32] noted that although the bacterial count was in the hundreds of millions per milliliter there was neither an odor nor taste to indicate this and it was not until putrefaction was under way that the milk appeared objectionable. There were constantly present bacteria that formed acid, others which acted on the protein, and still other neutral types; acid formers generally were in relatively smaller numbers than in milk held at higher temperatures but liquefiers were more numerous. Caseoses, amino acids, and probably peptones increased in the milk, apparently at the expense of the casein. Rate of digestion varied with different samples, but after 2 weeks was pronounced.

In milk at 0 C Ravenel *et al.*[17] found a great increase in plate count and in acidity. After 36 days there was an abnormal flavor and clumping of the casein; after 106 days there was gas formation, a bad odor, and a digested appearance. Chemical examination after 160 days showed that the total nitrogen had decreased, some apparently being carried away as free nitrogen in the gas that escaped, and that 72 per cent of the total nitrogen was in a soluble form, instead of the usual 20 to 25 per cent for normal milk.

Protein decomposition in milk or cream is very objectionable since it may result in formation of toxic products, and the possibility of these being produced constitutes a definite danger when milk or cream is held for extended periods at slightly above the freezing point. Certain cases of poisoning from ice cream probably have been due to long storage of the cream at approximately 0 C; at higher temperatures bacterial changes would have occurred much more rapidly but would have involved primarily carbohydrate decomposition, which ordinarily does not yield harmful products. It is probable that at 0 C 10 days is about the limit of safe storage, although much longer periods have been used repeatedly without harmful results.

Milk and cream in the range of 0 to 37 C (32 to 98.6 F). In general, in the range of 0 to 37 C, the higher the temperature at which milk or cream is held the sooner multiplication of organisms begins and the more rapid the rate; also, as the temperature increases there is a shift in the types of organisms that predominate. Low in the range *Pseudomonas* species and related psychrophilic types are relatively active because of their growth temperatures; they bring about various objectionable changes in flavor and odor. Higher in the range *S. lactis* largely dominates the fermentation, particularly when conditions of production have resulted in the presence of considerable numbers, because milk is such a favorable medium for it. Acid production by *S. lactis* tends to exclude organisms that are sensitive to acid but action of this species often is modified by growth of other types. Objectionable flavors may develop and *Escherichia-Aerobacter* species and other organisms may produce gas, even when acid formation by *S. lactis* is relatively rapid. At the higher temperatures lactobacilli may develop extensively and eventually cause high acidities.

Milk and cream above 37 C (98.6 F). Many organisms are capable of growth at temperatures above 37 C. Certain thermophilic species even grow at temperatures used in pasteurization; in some instances these organisms have been responsible for high counts on pasteurized milk, especially when pasteurization equipment has been used for successive lots of milk without sterilization between runs so that the organisms have become established in it. However, at temperatures not greatly above 37 C destruction of some species of bacteria begins.

Changes in numbers of bacteria in milk at some common holding temperatures. The increases that occur in numbers of organisms in milk held at 4.4 C (40 F), 10 C (50 F), and 15.6 C (60 F) are illustrated by the following data reported by Ayers *et al.*[33] as averages of a number of samples:

CHANGES IN NUMBERS OF BACTERIA IN MILK AT VARIOUS TEMPERATURES

Cows Clean and Bedded, Small Top Pails, Sterile Utensils (Average 20 Samples)

Temperature of Holding	Bacteria per Milliliter				
	Fresh	*24 Hours*	*48 Hours*	*72 Hours*	*96 Hours*
4.4 C	4,295	4,138	4,566	8,427	19,693
10.0 C	4,295	13,961	127,727	5,725,277	39,490,625
15.6 C	4,295	1,587,333	33,011,111	326,500,000	962,785,714

Cows Dirty, Manure Removed Twice Weekly, Utensils Not Sterile (Average 30 Samples)

Temperature of Holding	Bacteria per Milliliter				
	Fresh	*24 Hours*	*48 Hours*	*72 Hours*	*96 Hours*
4.4 C	136,533	281,646	538,775	749,030	852,835
10.0 C	136,533	1,170,546	13,662,115	25,687,541	41,207,272
15.6 C	136,533	24,673,571	639,884,615	2,407,083,333	5,346,666,666

From these results it is evident that at 4.4 C growth is comparatively slow, at 10 C it is considerably more rapid, and that the difference between 10 C and 15.6 C is very striking. The following data, also reported by Ayers *et al.*[33] compare growth at 15.6 C and 21.1 C (70 F):

CHANGES IN NUMBERS OF BACTERIA IN MILK AT 15.6 C AND 21.1 C

Clean Milk (Average 16 Samples)

Temperature of Holding	Bacteria per Milliliter			
	Fresh	*12 Hours*	*24 Hours*	*48 Hours*
15.6 C	3,243	4,056	123,562	26,176,923
21.1 C	3,243	19,312	10,006,875	2,014,692,307

High-Count Milk (Average 13 Samples)

Temperature of Holding	Bacteria per Milliliter			
	Fresh	*12 Hours*	*24 Hours*	*48 Hours*
15.6 C	707,761	3,376,961	48,550,923	296,100,000
21.1 C	707,761	6,608,846	221,916,666	1,853,000,000

In these data also a small temperature difference resulted in considerable difference in rates of multiplication of bacteria.

The above tabulations indicate that the range from 10 C to 21.1 C is particularly important from the standpoint of influence on rate of multiplication of bacteria and, accordingly, on the time of appearance of undesirable changes; they also suggest that, on farms where this range largely is involved because of the use of water for cooling, it is especially important that the cooling be as complete as possible and carried out quickly. The data further indicate that rate of multiplication of the bacteria is influenced by the quality of the milk and that the increase in organisms, on a percentage basis, is less in clean milk than in high-count milk. This is to be expected since, with clean milk, a considerable portion of the organisms come from the interior of the udder and, accordingly, are species that grow comparatively slowly, whereas, with high-count milk, many of the organisms come from external sources and include species that multiply rapidly. This difference between clean and high-count milk explains why conditions that are reasonably satisfactory in preventing changes in certain lots of milk or cream are unsatisfactory for other lots, and also why conditions of production have a greater influence on the quality of milk or cream after holding than might be expected from the actual number of bacteria introduced by the careless methods.

Holding milk and cream on farms. Holding milk or cream for varying periods is necessary on practically all dairy farms. It is most satisfactorily accomplished by entirely preventing growth of organisms but this is not always possible because the low temperatures required are not available. Two steps are involved: (1) rapid cooling to suitable temperatures, and (2) maintenance of such temperatures during the holding. Use of refrigeration equipment simplifies these steps and is becoming more and more common.

Ice can be used successfully to cool cans of milk or cream in a tank of water, provided sufficient amounts are employed, but the expense and inconvenience of ice, even when it is available for the harvesting, are such that it is rapidly being replaced by mechanical refrigeration; moreover, there is a definite tendency to use too little of it because of the work involved. Well and spring water are advocated for cooling and, if cold enough and in sufficient amounts, make possible the keeping of milk and cream in a satisfactory condition for a reasonable period. Tanks of water can be conveniently maintained at satisfactory temperatures with refrigeration equipment.

Price *et al.*[34] noted that, in water at 1.7 to 4.4 C (35 to 40 F), milk in 10-gal cans cooled from 35 to 15.6 C (95 to 60 F), or below, in 1 hr and to 13.3 C (56 F) in 2 hr; when the milk was precooled with a tubular surface cooler to 19.4 C (67 F), it cooled to 12.8 C (55 F) in 1 hr and to 11.1 C (52 F) in 2 hr in the water. With water at 1.7 C circulated around the cans, milk in 10-gal cans cooled from 35 C to below 10 C (50 F) in 1 hr and to 5.6 C (42 F) in 2 hr; when the milk was precooled with a tubular cooler to 19.4 C, it cooled to 6.7 C (44 F) in 1 hr and to 5.6 C in 2 hr in the circulating water. The bacterial content of milk held in 10-gal cans in water at 1.7 to 4.4 C did not increase during the first 12 hr. Stirring milk in 10-gal cans in water at 1.7 to 4.4 C did not materially increase the rate of cooling.

Marquardt and Dahlberg[35] found that, when milk above 32.2 C (90 F) was placed in water at 1.7 to 4.4 C and stirred after 1 and 2 hr, the temperature was around 15.6 C in 1 hr and at 10 C in 2 hr; with the milk at 21.1 to 26.7 C (70 to 80 F) as it was placed in the tank, it dropped to 10 C within 1 hr. Thermometers set in fixed positions in an unstirred can of milk and read at 10-min intervals for 4 hr indicated that the milk cooled rapidly at the sides and in the center at a point near the handles and, presumably, more rapidly below this; however, 1 in. below the neck and in the center of the can, cooling was so slow the milk reached 15.6 C only after 4 hr.

According to Anderson,[36] cooling milk in a tank with water above 10 C and without agitation of the water did not afford adequate protection against bacterial growth; he concluded that fresh milk should be cooled entirely and quickly to below 10 C, preferably to 4.4 C, to prevent growth. Anderson and Nicholas[37] reported that cooling milk with well or spring water in a tank without agitation of the water is not satisfactory for production of a high-quality product unless the water is approximately 10 C, or lower, and unless that temperature is maintained during the entire cooling period; they suggested running water should maintain a temperature of 8.9 C (48 F), or below, to cool fresh milk efficiently.

In some instances attempts are made to cool milk and cream by exposing cans of the products to cold air, for example out-of-doors or in an unheated room in winter, or in a refrigerated room. Air conducts heat from the cans only very slowly and adequate temperature drops are greatly delayed. Roberts and Larson[38] reported that after 8 hr in a dry box cooler at 5 C (41 F), milk in the tops of 10-gal cans was above 21.1 C; they concluded that cold air is practically worthless for cooling milk in 10-gal cans.

When cold air is in active motion its cooling effect definitely is greater than when it is quiet. Although exposure of milk or cream in cans to air in active motion at relatively low temperatures results in slow cooling, exposure for extended periods may result in freezing. Because of this danger, producers in certain areas do not take advantage of the natural refrigeration available and in winter even hold milk or cream in a comparatively warm place to insure against freezing.

The value of electric refrigeration in maintaining the temperatures needed to hold milk or cream on farms is now generally recognized as a result of various investigations.[34,35,36,37,39] In many important dairy areas well water is not cold enough to control bacterial growth in milk. Where it is available, it frequently is used in insufficient amounts because of the difficulty or cost of pumping. Adequate springs now are rare. Ice is inconvenient and often expensive.

Nicholas and Blasingame[39] noted that milk will cool more rapidly and uniformly in farm electric coolers when the water bath is agitated and that the agitation should continue for at least 1 hr. They found a can of milk will cool more in the first hour than in the 11 succeeding hours. Under the same thermostat limits, milk will cool more rapidly with a water to milk ratio of 5 than with a ratio of 3.

Abraham and Outwater[40] concluded that mechanical refrigerators are the most effective means of cooling milk on farms and probably the most economical, except where an adequate supply of cold spring water at 10 C, or less, is readily available, and also that unless a producer is equipped with a thermostatically controlled electric pump and has an ample supply of well water at 10 C, or lower, use of well water alone is not dependable in most cases.

With the production of large volumes of milk on a farm, cooling in cans becomes impractical and milk coolers with mechanical refrigeration are essential. The milk can be put through the cooler as it is drawn and then run into cans or it can be run into an insulated tank from which it is pumped to a tank truck for transportation to the milk plant.

When evening milk is held overnight on the farm and delivered with the fresh morning milk, it often has a higher bacterial count than the morning milk and, in some instances, the difference is striking. The large numbers of bacteria in many lots of milk held on farms are due to slow and incomplete cooling. This is indicated by the temperature of the milk at delivery and by the fact that, with proper cooling, evening milk is no higher in bacteria at delivery than fresh morning milk. On farms using ordinary production methods, contamination of morning milk often is greater than that of evening milk because of the condition of the stable and cows and the hurried treatment given the utensils following the evening milking.

Dearstyne and Jones[41] found great variations in the bacterial contents of evening and morning milk supplied to various dealers and pointed out the importance of this in grading milk on the basis of numbers of contained bacteria. Macy[42] studied, under summer conditions, the growth of bacteria in uncooled morning milk from the time of production until delivery at the pasteurizing plant and noted regular increases, although in the samples with low initial counts the final counts also were low. He concluded that in areas where conditions can be satisfactorily controlled the farmer might be permitted to deliver uncooled morning milk to the pasteurizing plant but that, as a general practice in all communities, it is not to be recommended.

With twice a day delivery of milk for manufacturing purposes, there sometimes is gross carelessness in its handling. Producers and receivers may fail to recognize that with long truck routes, etc., 4, 6, or even 8 hr may elapse between milking and delivery of the milk and that without cooling of the product there may be extensive growth of bacteria in it. The interval between milking and cooling or processing of the milk is the important consideration, not the number of deliveries per day.

Effect of delayed cooling. From studies on the influence of delayed cooling on the quality of milk, Frayer[43] concluded that prompt cooling to 10 C (50 F), or less, is a vital factor in producing good-quality milk which will keep well and that damage to the future quality of milk is roughly proportional to the period elapsing before cooling. He pointed out that damage to the future quality is not always fully apparent in milk as received at the plant. Frayer[44] later reported that cooling milk immediately to 10 C is a fairly satisfactory procedure and that cooling morning milk to 15.6 C (60 F) is fairly satisfactory, but that cooling milk to be held overnight on the farm to this temperature is unsatisfactory. Cooling milk immediately to 4.4 C (40 F), or lower, and holding it there seemed to produce the best results in respect to its quality, both present and future. Fresh milk that was not cooled deteriorated progressively for each hour of such delay and the lower the initial temperature the better. In studies on pure cultures inoculated into milk at 23.9 C (75 F), Frayer[45] found that the effect of delayed cooling varied widely with different species. The various delays before the milk was cooled gave average increases in bacterial counts as follows: 2 hr, 22 per cent; 4 hr, 221 per cent; 6 hr, 1,230 per cent; 8 hr, 6,229 per cent.

Concentration of bacteria in fat layer. When milk is allowed to stand, the fat concentrates in the surface layers and brings with it many of the bacteria, so that the numbers per milliliter definitely are larger than in the lower layers. This is shown by the following data reported by Marquardt and Dahlberg:[35]

CONCENTRATION OF BACTERIA AND FAT IN TOP OF MILK ON HOLDING

Kind and Age of Milk	Bacteria per Milliliter at Initial Temperature of		Fat Content at Initial Temperature of	
	36.7 C (98 F)	*10 C (50 F)*	*36.7 C*	*10 C*
Fresh milk mixed	53,000	53,000	5.0	5.0
Top milk 1 hr old	133,000	108,000	14.7	7.7
Top milk 2 hr old	195,000	167,000	17.9	15.3
Top milk 5 hr old	282,000	262,000	19.8	16.4
Mixed milk 6 hr old	52,000	53,000	5.0	5.0

Anderson[36] noted that in the majority of instances 90 per cent, or more, of the organisms in a 10-gal can of milk cooled 12 hr are in the cream layer. Nicholas and Anderson[40] found the ratio between the number of organisms in fresh milk and in the cream layer of cooled milk varied from as low as 1 to 5.5 to as high as 1 to 173. After cooling, homogenized milk apparently exhibited a constant butter-fat and bacterial content at all levels in a can.

With adequate cooling of milk being held on farms, concentration of bacteria in the cream layer probably is of no practical significance. When the milk is mixed, they are redistributed to give the product essentially the same count as it had originally. Without adequate cooling, concentration of organisms in the cream layer may result in more rapid changes there than in the lower layers. For example, ropiness in the cream layer of a can of milk apparently is influenced by the concentration of organisms there, although such factors as air supply also may be involved.

Transportation of milk from farms. Transportation of milk in cans from farms to receiving stations or plants involves a number of considerations. Open trucks, or trucks covered only with a tarpaulin, offer little protection against collection of dust, insects, and other material on can lids, lips, etc., from which they can fall into the milk on dumping; also, there is little protection against temperature changes. Properly closed trucks protect against the collection of extraneous matter on cans; if insulated, they protect against both the heat of summer and freezing winter temperatures. Closed trucks may delay loading and unloading, particularly if large loads of cans are being hauled, but they are essential to adequate handling of milk in cans and are being demanded by more and more regulatory officials.

Poorly cooled or uncooled cans of milk may significantly increase the temperature of cans of well-cooled milk near them. Anderson[36] found that, during a transportation period of about 2 hr, cooled milk at 3.4 to 7.8 C (38.1 to 46.0 F) in cans protected only by a tarpaulin showed temperature increases of 7.2 to 11.9 C (12.9 to 21.5 F). The increases were dependent on two factors, air temperatures of 15.4 to 21.6 C (59.8 to 70.8 F) and proximity of warm cans of morning milk.

In attempts to improve the quality of milk received at plants, transportation of the product under adequate conditions should be included. It should be protected as completely as possible against entrance of extraneous matter and against significant development of organisms.

Cooling small lots of cream intended for butter. The butter industry uses a great deal of sour cream, much of which is produced on farms having only a few cows. It is shipped directly to the butter plants or collected in cream stations from which it goes to the plants. If given satisfactory care, the cream yields an average grade of butter that is very acceptable to many consumers.

Because the farms produce only small volumes of cream, deliveries are infrequent and its care must be inexpensive. Cold, running water is very useful in cooling and holding the cream, even if it is not as cold as is needed to prevent bacterial growth in sweet milk. Other methods include the evaporation method, in which water is evaporated from wet sacks or other wicking material surrounding the cans, and the spray method, in which water is sprayed over the cans. Mechanical refrigeration is preferred; however, holding raw cream for excessive periods with such refrigeration may result in very objectionable odors and flavors because of the action of psychrophilic bacteria and milk lipase.

Price *et al.*[47] reported that cream cooled and held in a tank filled with water at 15.6 C (60 F), the water being changed twice daily, remained sweet for 18 hr but was sour after 42 hr with an acidity of 0.4 to 0.5 per cent; with the water at 10 C (50 F), the cream had an acidity of 0.20 after 42 hr and an acidity of 0.41 after 66 hr. Cream held in an ordinary ice refrigerator at 7.2 C (45 F) cooled more slowly for the first 7 hr than when held in water at 10 C; it had an acidity of 0.19 per cent after 42 hr and of 0.23 after 66 hr. Cream cooled in an electric refrigerator having dry storage remained sweet for twice-weekly shipments, both with natural and forced air circulation. Cream cooled in refrigerated water at 1.7 to 4.4 C (35 to 40 F) dropped from 26.7 to 13.3 C (80 to 56 F) in 1 hr and remained sweet and of good quality for 90 hr.

The Department of Agriculture, Dominion of Canada,[48] reported the following acidities for cream held at different temperatures for different periods:

ACIDITIES OF CREAM HELD AT DIFFERENT TEMPERATURES FOR DIFFERENT PERIODS

Period Held (Hours)	Average Temperature of Cream	Average Acidity of Cream (Per Cent)
36	10.3 C (50.6 F)	0.145
36	12.8 C (55.0 F)	17
36	14.2 C (57.5 F)	.19
36	14.7 C (58.5 F)	.21
36	17.8 C (64.0 F)	.51
60	11.7 C(53.0 F)	.15
60	13.1 C (55.5 F)	.51
72	14.8 C (58.7 F)	.38
84	8.6 C (47.5 F)	.165
84	12.2 C (54.0 F)	.39

It should be recognized that holding temperature is not the only factor determining the changes in cream held for buttermaking; the numbers and types of organisms in the cream originally also are important and excessive contamination may cause very rapid deterioration.

Martin *et al.*[49] found that efficiency of the evaporation method was not influenced by the type of wicking and that rate of cooling was approximately the same when the water was drawn by capillarity from the top of a can as when it was drawn from the top and bottom. Circulation of air in the room with a small fan increased the efficiency. Rate of cooling was greatly increased with a decreased relative humidity. Efficiency of the spray method was affected most by temperature of the water and rate of delivery. With this system, cans with or without a sack covering were cooled equally, showing that evaporation plays a relatively minor part in spray cooling. Under the usual summer conditions in Kansas, the spray system commonly was more efficient than the evaporation system. If the water available for spraying is at 18.3 C (65 F), or lower, the cooled cream should be below 21.1 C (70 F). If the air is above 29.4 C (85 F) and the relative humidity is in excess of 50 per cent, the evaporation method cannot be expected to cool cream below 21.1 C. While temperatures such as 18.3 and 21.1 C permit large increases in numbers of organisms on relatively long holding, they are better than still higher temperatures in that they favor less objectionable organisms and have some effect on the rate of multiplication.

In the cream stations or in the plants, it is essential that the sour cream be cooled quickly and held cold. This is best achieved with mechanical refrigeration. The product contains enormous numbers of organisms that will produce objectionable changes very quickly if permitted to grow so that adequate cooling is essential. In the plant the cream should be processed as soon as possible.

Care of milk and cream reaching dairy plants. When uncooled or inadequately cooled milk or cream is received at a plant, it should be processed at once or properly cooled for holding. Dairy plants are so regularly equipped with mechanical refrigeration that under normal conditions adequate holding temperatures for milk and cream can be quickly reached with coolers or vats. In some instances an uncooled product is held without cooling until additional deliveries are received so that processing can begin; such a practice not only harms the milk but encourages the observant producer to think that cooling is not important. Often milk which comes to a plant uncooled is in such a condition microbiologically that growth of organisms and changes in flavor and general quality can be very rapid.

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Chapter 7

Milk Enzymes

Various enzymes normally are present in milk. Those which have been studied most are protease, lipase, peroxidase, catalase, and phosphatase. Protease is important in the ripening of certain cheeses; lipase causes changes which usually are objectionable, although with some dairy products they may contribute to the desirable flavor; and peroxidase, catalase, and phosphatase are the bases of certain tests used on milk and its derivatives. Other enzymes reported in milk include amylase, lactase, mono-butyrinase, reductase, and salolase. In certain instances lipase quickly produces rancidity in raw milk and cream but, ordinarily, the enzymes of milk do not bring about changes rapidly and are of little importance in its keeping qualities.

The enzymes of milk require consideration in connection with the bacteria in dairy products because of the similarity of enzymatic and bacterial changes in certain of the constituents of milk, and because both are greatly influenced by temperature.

Differentiation of changes due to enzymes and to organisms. In the study of the changes due to enzymes, action of organisms commonly is prevented by a reagent, such as chloroform, ether, or formaldehyde. It is advisable to examine, both culturally and microscopically, the material under investigation to be certain that organisms are not developing. The importance of this was shown through the isolation by Babcock and Russell [1] of an organism that possessed a definite ether tolerance; the character was lost during a series of transfers on agar.

Rahn [2] pointed out that the changes produced by enzymes and by bacteria differ in rate. With enzymes the active agent does not increase and, accordingly, the rate of change does not but tends to fall off with time, whereas with bacteria the active mass increases and, as a result, the rate of change does also until conditions become unfavorable for the organisms. In impure culture the influence of one species on another may greatly modify the changes occurring.

Bacteria sometimes influence the action of enzymes through their effect on the reaction. This is illustrated by the slower action of protease in an acid than in a neutral or alkaline medium.

PROTEASE

In 1897, Babcock and Russell[3] reported that in milk to which chloroform or ether had been added coagulation and an increase in soluble nitrogen eventually occurred. Their experiments indicated that precipitation of the casein was influenced by the temperature of holding more than anything else. They made enzyme preparations that had a curdling as well as a digesting action on milk. Babcock *et al.*[4] proposed the name galactase for the coagulating and digesting enzyme, and presented data suggesting that the curdling action is independent of the peptonization. Their results further show that activity of the enzyme is materially retarded in the presence of acid and this was interpreted as indicating relationship to the tryptic rather than the peptic type of enzyme. The name galactase is not in agreement with the present system of enzyme nomenclature and protease is the preferred designation. Babcock *et al.*[5] studied protease in comparison with various enzymes and cultures of bacteria from the standpoint of the decomposition products in milk. They concluded that the products formed by protease approach more nearly those formed by the peptonizing bacteria than by any of the enzymes of animal origin.

Protease can be demonstrated in milk by treating a sample with chloroform or ether, stoppering tightly to prevent loss of the reagent, and then holding at a favorable temperature, such as 37 C (98.6 F). The milk usually forms a soft curd in 1 to 3 weeks; the curd gradually acquires a somewhat transparent appearance, owing presumably to the proteolysis taking place. Soluble nitrogen determinations on the fresh and curdled milk show the extent of the decomposition. Protease also can be demonstrated at 21.1 C (70 F) but it is less active there than at 37 C.

The presence of protease has been noted in milk of various species,[6] including man, sheep, goat, pig, horse, burro, and a half-breed buffalo. During the separation of cows' milk, protease[7] is highly concentrated in the slime and slightly concentrated in the cream. The enzyme is active[4] in the presence of chloroform, ether, benzene, or toluene but mercuric chloride, formaldehyde, phenol, or carbon bisulfide destroy it; 1 per cent chloroform[7] in skim milk or buttermilk was found to inhibit proteolysis entirely, whereas smaller amounts permitted it. Proteolysis is more active in slightly alkaline or neutral reaction but also occurs when some acid is present. Protein decomposition brought about by protease goes to the amino acid stage.

Babcock *et al.*[4] found that heating enzyme solutions 10 min at 76 C (168.8 F) destroyed protease, and that 10 min at 71 C (159.8 F) materially reduced its activity. Rogers *et al.*[8] reported that with flash pasteurization protease was much weakened by temperatures between 71 and 77 C (170.6 F) but was not destroyed at 93 C (199.4 F), the highest temperature employed. Protease evidently is the most resistant to heat of the enzymes normally in milk.

Protease appears to be an important factor in the ripening of various hard cheeses, its role being to aid in the breakdown of the complex nitrogenous constituents. This decomposition is necessary for the proper development of texture and flavor, and chemical examinations show that it may involve a considerable portion of the protein originally present. Because of the curd in butter, action of protease also must be considered as a possible factor in the deterioration of this product. Thatcher and Dahlberg[7] obtained a uniform and measurable proteolytic action from two samples of fresh dairy butter but none from fresh creamery butter. Stored dairy butter and stored creamery butter gave greater increases in soluble nitrogen than the fresh butter. The investigators noted that sodium chloride greatly influenced the activity of protease, 15 per cent in skim milk entirely preventing proteolysis, which led them to conclude that protease cannot act in normal salted butter because of the salt content.

Warner and Polis[9] found that in solutions of casein an enzyme caused slow hydrolysis, a decrease in viscosity, and appearance of nitrogenous products soluble at *p*H 4.6 and in trichloroacetic acid. Presumably, the enzyme is the same as that studied by Babcock and associates. It was present in all samples of commercial casein and in all laboratory preparations made by the usual methods; since it was present with either acid or rennet precipitation, it would be present in cheeses. Optimum activity was at *p*H 8.5; it was active at *p*H 5.8, which was the lowest *p*H at which satisfactory solutions could be prepared, and at *p*H 11.2.

LIPASE

Lipase hydrolyzes fat to free fatty acids and glycerol. In milk and other dairy products lipase activity may result in accumulation of appreciable amounts of free fatty acids and then a rancid flavor commonly is evident. Volatile fatty acids are of most importance in this connection and butyric acid appears to be of special significance because of its conspicuous odor, although it makes up only a small part of the total fatty acids. Lipase activity also may be the cause of off flavors in milk that are not definitely recognized as rancidity. Ordinarily, lipase activity is objectionable in a dairy product, but under certain conditions, notably in the ripening of blue-veined cheeses and in the preparation of milk derivatives for use with chocolate, it is desirable.

Lipase in milk is associated with the serum and, accordingly, acts at the surfaces of the fat globules. The amount in milk varies and in addition its activity is greatly influenced by various factors so that certain samples of raw milk slowly or rapidly become rancid, whereas others do not. Milk from cows advanced in lactation sometimes becomes rancid so quickly that it is practically unusable in a raw condition, either as milk or after conversion to butter. Rancidity due to lipase activity is not confined to the milk of cows in advanced lactation and occurs with milk from certain animals in middle or early lactation. In some cases udder infections may be responsible; in others there is no evidence of any abnormality. Certain data [10] indicate a relationship between the estrus cycle and lipase activity in the milk.

Hileman and Courtney [11] reported there are two factors which govern the amount of lipase secreted in the milk. One is the lactation cycle, the amount of lipase increasing as lactation is prolonged; nature of the other is not known. They noted that the lipase content of milk produced in New York is at a minimum in June or July and reaches a maximum in December or January. Tarassuk [12] worked with a Holstein cow that produced milk in which lipase was naturally active and found it possible to obtain milk high in lipolytic activity or milk free from it by changing from a dry feed to a green feed. The level of carotene [13] in blood or milk fat was not by itself the cause of a cow secreting milk which spontaneously developed rancidity.

Methods of estimation. Various methods and modifications have been used to detect lipase activity in dairy products. One procedure is to allow the material being tested to act on a simple triglyceride (such as tributyrin), or on fat or oil, in some medium such as milk or a buffer solution.[14] The mixture is titrated initially and after holding, using alkali and an indicator, often under special conditions; or two aliquots are prepared in one of which (control) the lipase is destroyed by heat. Holding conditions and *p*H should be standardized. A significant increase in acidity indicates hydrolysis. With long holding, chloroform or toluene may be needed to prevent bacterial growth.

At one time formaldehyde was used to prevent growth of organisms in lipolysis tests. However, it appears that this compound limits lipolysis.[15] With a series of compounds tested,[16] it was the only one that inhibited lipase; chloral hydrate, toluene, merthiolate, sodium fluoride, and chloroform did not.

Peterson *et al.*[16] developed a procedure in which skim milk, obtained by centrifuging the sample to be studied, is tested for its ability to hydrolyze tributyrin at a *p*H of 8.5, which was found to be optimum for cows' milk lipase. The results are obtained with a special titration procedure and can be calculated to lipase units.

Cream has been employed as a substrate in lipase tests. Rice and Markley[17] used a high-fat cream and added sufficient sucrose to saturate it and any water included with the material to be tested in order to prevent action of organisms; 2 parts of sucrose to 1 part of water were used, and the mixture of cream and sugar boiled to facilitate solution and to destroy enzymes in the cream. The material suspected of containing lipase then was added and titrations made at once and again after holding. In some cases the cream used in testing for lipase is homogenized in order to increase the fat surface of the system and thus facilitate lipase action. Hileman and Courtney[11] studied the lipase content of milk by separating it, standardizing the cream at 40 to 42 per cent fat, and following the fat hydrolysis by titrating; in some trials low temperatures were used to prevent bacterial action and in others the cream was saturated with sucrose. With this procedure concentration of the lipase is relatively high since there is no dilution with heated cream; if the sucrose is omitted, the fat exists in a normal state and the conditions then are like those in commercial raw cream.

Lipase activity has been measured by titrating the free acid developed in the fat phase of milk.[18,19] The milk commonly is held at a low temperature for a short period and then churned and fat recovered and titrated. This method also can be applied to fat recovered from other products.

Kelly[10] developed a method in which the milk to be tested was first dried; one volume was extracted by mixing at room temperature twice with acetone, once with equal parts of acetone and ether, and twice with ether, each time using 5 volumes of solvent. A soft, fine precipitate was obtained which kept indefinitely; it contained only 5 or 6 per cent moisture and so the dried milk and substrate were used alone without preservative. Various substrates were employed, including sterilized butter oil. At first, definite weights, usually 4 gm samples of dried milk, were saturated with the pure substrate but later the substrate was used as a 20 per cent solution in ether; the ether then was evaporated leaving a dry, powdery sample. Lipase activity was measured by the titration value obtained under definite conditions.

Certain investigators have studied lipase activity in milk by the reduction in surface tension that results from the products of fat hydrolysis.

The effect of temperature on lipase activity should be recognized. In a comparison of 3 to 4, 27, and 37 C (37.4 to 39.2, 80.6, and 98.6 F) Roahen and Sommer[15] found lipolysis was most extensive at 37 C, slightly less at 27 C, and decidedly less at 3 to 4 C.

Frequency of rancidity. A rancid flavor rather frequently develops in milk from individual animals. Roadhouse and Henderson[20] collected samples from 536 cows in 12 herds, many of the cows being in advanced lactation. When examined after holding for 6 hr at room temperature, 17 (3.17%) of the samples were rancid. Five herds yielded no rancid samples and the others yielded from 1.5 to 23.5 per cent that became rancid. In studies on 12 to 20 animals over a 140-week period, Fouts and Weaver[21] observed rancid flavors with alarming regularity in milk from 3 to 5 animals. Rancidity was most common near the end of lactation but occurred almost any time in the milking period. It was least noticeable during spring and summer when the cows were on pasture. In over 5,000 observations, 10.4 per cent of the criticisms indicated some degree of rancidity. Krienke[22] noted that a high percentage of the cows in the herd examined produced rancid milk during the period studied. Some cows were very persistent in producing it; others produced it occasionally and at irregular intervals. Several cows produced rancid milk at the beginning of lactation and others produced it only at the end. It was produced by some cows at the beginning and end of lactation, with an intermediate period during which the defect was absent. A few cows produced the defect during the second lactation when it had not been produced during the first, and several cows produced it during the initial lactation but not during the succeeding one. Some cows produced the defect during successive lactation periods.

Using a direct determination of free acid in the fat phase, Herrington and Krukovsky[19] found that lipolysis occurs in practically all samples of raw milk and that there is an appreciable increase in free fatty acids between milking and delivery of the milk to a pasteurizing plant. As drawn from the udder, there is only a slight variation in the acidity of the fat in the milk of different cows, but after a few hours there may be a wide variation. Lipolysis was retarded by sufficiently rapid cooling. The authors noted that milk contains at least two lipases. One is completely inhibited by very small amounts of formaldehyde; the other, apparently, is not sensitive to moderate amounts.

Tarassuk and Henderson [23] reported that rate of lipolysis of milk fat due to a naturally active lipase did not follow the rate of dilution of the enzyme with normal milk. Development of rancidity in raw milk by a naturally active lipase could be prevented by mixing with normal milk within 1 hr of milking. The amounts to be mixed depended on the lipase concentration; a proportion of active and normal milk of 1 to 4, or higher, always prevented rancidity. To ensure effectiveness of the method, mixing must be done within 1 hr of milking, either before cooling or immediately after.

Factors influencing activity. Size of the fat globules is an important factor in lipase activity. With small globules the surface area is relatively large and lipase activity is facilitated. Milk that is homogenized without pasteurization quickly becomes rancid and homogenized milk is sold only in a pasteurized condition. Dorner and Widmer [24] found that when milk from individual animals was homogenized and held at 20 C (68 F), the period required for rancidity to develop varied widely.

Plants shipping raw cream sometimes encounter serious difficulties through deveolpment of rancidity in the product. According to Sharp and de Tomasi,[25] a number of plants have found that when the milk was heated at 37.8 to 46.1 C (100 to 114.9 F) and then cooled and separated at 23.9 to 26.7 C (75 to 80 F) the off flavor did not develop, whereas without heating previous to separation the defect appeared. The heating did not destroy the lipase because when the cream was homogenized it developed the flavor as rapidly as the unheated cream.

Krukovsky and Sharp [26] noted that shaking raw, whole, cows' milk, with the fat in the liquid or partially liquid state, induced lipolysis which continued after the milk had been cooled to low temperatures. The effect of shaking was attributed to an alteration in the surface characteristics of the fat globules that created a condition more favorable for lipolysis. The authors considered that all milk is capable of appreciable lipolytic activity if subjected to suitable treatments but that only some cows, particularly those in advanced lactation in winter, secrete milk which will show natural lipolytic activity when cooled and held. Hlynka *et al.*[27] reported that milk which was churned 15 min at 30 C (86 F) showed fat dispersion roughly equivalent to that of 20 per cent homogenized milk and concluded that the increase in the fat-aqueous interface due to fat dispersion may largely explain the mechanism of increased lipase action in agitated milk.

Krukovsky and Herrington [28] found that lipolysis in cold milk was accelerated by warming the milk slightly and recooling. For maximum increase in lipolytic activity, milk was precooled to 5 C (41 F), or lower, rewarmed to 30 C (86 F), and then recooled below 10 C (50 F). The period the milk is held at the pre-cooling temperatures seemed to be less important than the actual temperatures employed, at least with moderate rates of heating and cooling. Rate of lipolysis seemed dependent on the crystalline state of the fat and, consequently, on the previous history of the milk. Differences in history were retained, even at 20 C (68 F), for at least 22 hr. With 61 cows,[29] much variation was noted in rates of lipolysis with different samples of milk and in response of the samples to warming and cooling. There was no apparent correlation between rates of lipolysis and stage of lactation, stage of gestation, or milk production. Most of the samples contained both the formaldehyde-tolerant and formaldehyde-sensitive enzymes; sometimes one predominated and sometimes the other. In the trials of Peterson *et al.*[16] warming milk from 0 to 30 C (32 to 86 F) over a period of 45 min, then recooling to 0 C over a period of 60 min, had no effect on lipase activity.

Krukovsky and Sharp[30] noted that the lower the temperature required for crystallization of the fat fraction, the greater the increase in acidity when used as a substrate for milk lipase; this indicates the rate of lipolysis is dependent on melting point of the fat or on degree of solidification of the fat at a given temperature. Resurfacing the fat globules as a result of emulsification with skim milk rendered the fat more susceptible to action of lipase.

Davies[31] found that traces of heavy metals in amounts up to 25 ppm have the property of inhibiting lipase action in butter from unpasteurized sweet cream; degree of inhibition varied with the metal and its concentration. Copper was the most inhibitory of the metals studied; iron, nickel, cobalt, manganese, and chromium were less active; tin and aluminum had no effect. According to Krukovsky and Sharp,[32] normal milk lipase is susceptible to inactivation by dissolved oxygen, and this inactivation is accelerated by heat and by dissolved copper. Dissolved copper caused no inactivation of lipase in normal milk in the absence of dissolved oxygen. Hlynka and Hood[33] reported that cysteine can reverse the inactivation of milk lipase brought about by aeration or aeration and copper. Anaerobic environment may bring about some reversibility of oxidatively inactivated milk lipase, but not in the presence of copper. Hlynka *et al.*[34] noted that, although copper inhibits lipase activity in milk under ordinary conditions, it is ineffective as a lipase inhibitor in cheddar cheese; this was attributed to the reducing potential of the cheese.

Castell[35] found that addition of small amounts of ammonia to milk held at low temperatures accelerated reduction of the surface tension and hastened development of rancid flavors.

Lipase in cream. Roahen and Sommer[15] reported that gravity-separated cream showed appreciably greater lipolytic activity than separator cream of the same fat content, loss of lipase in the separator slime, presumably, being a factor. Cream separated at 43.3 C (110 F) showed less lipolysis than cream separated at 23.9 C (75 F); similarly, the 43.3 C skim milk caused less lipolysis than the 23.9 C skim milk.

Krukovsky and Sharp[30] noted that total lipolytic action increases with fat content up to 35 to 45 per cent, but acidity per unit of fat or acidity per unit of plasma increases with fat content up to 8 to 10 per cent and then remains constant or decreases.

Cream in which lipase has been active commonly is difficult to churn. Palmer[36] suggested that this is due primarily to excessive foaming which accompanies formation of soaps from the fatty acids liberated. Krukovsky and Sharp[37] noted that the difficulty of churning and the foam formation in cream from cows in advanced lactation probably are due largely to the lipolytic action and concentration of the resultant soaps and fatty acids in the air-plasma interface; pasteurization of the milk or cream as soon as it was drawn largely prevented the difficulty in churning. Fouts and Weaver[21] found that cream from rancid milk samples required 10 to 500 per cent longer to churn than cream from normal samples.

Inactivation by heat. In the investigations of Rogers[38] action of milk lipase was materially weakened by an exposure of 10 min at 45 C (113 F) and was entirely destroyed at 60 C (140 F) for this period. Rogers *et al.*[8] found that with the continuous method of pasteurization lipase was destroyed at 70 C (158 F). Doan[39] heated milk of different fat contents under various conditions and homogenized it immediately at 2,000 lb pressure; it then was tested for lipase activity by titration. Flash heating at about 63.9 C (147 F), 15 min at about 56.7 C (134 F), or 30 min at about 55.6 C (132 F) prevented rancidity in the milk when it was held at 1.7 to 4.4 C (35 to 40 F) for 24 hr. On the basis of *p*H or surface tension changes as indicators of lipase activity, essentially the same exposures were required to inactivate the enzyme. Nair[40] reported lipase could not be detected in whole or skim milk powder desiccated by a drying system in which the fluid milk had undergone preliminary pasteurization at 63 to 64.5 C (145.4 to 148.1 F) for 30 min.

Trials by Tarassuk[12] indicated that lipase was partially inhibited by heating milk at 43.3 C (110 F) for 30 min and completely inhibited at 54.4 C (130 F) for 30 min. Krukovsky and Herrington[41] churned unsalted butter from fresh cream held at various temperatures for 0 to 150 min and measured the rate of lipolysis by titration of free acid in the fat. At 43.3 C lipolysis was first activated and then reduced as the holding increased; the rate was reduced about two-thirds by holding 150 min. At 51.7 C (125 F) the rate of lipolysis was reduced about one-half after 20 min (estimated) but still was measurable after 150 min. At 60 C (140 F) the rate of lipolysis was reduced more than one-half at 0 holding time; it was measurable with 15 min holding but not with 35 min. At 68.3 C (155 F) the rate of lipolysis was scarcely measurable after 0 min holding.

Lipase in mammary tissue. Kelly[42] found that tissue from bovine mammary glands not developed by pregnancy contained only traces of active lipase, whereas tissue from developed glands contained significant amounts.

Inhibition of *S. lactis* by rancid milk. Tarassuk and Smith[43] noted the inhibitory effect of rancid milk on growth of *S. lactis* and showed that this was due to the low surface tension. Appreciable growth of *S. lactis* in rancid milk increased the surface tension and under optimum conditions this increase yielded a final surface tension value approaching that of normal milk. The change in surface tension apparently results from utilization of surface-tension-lowering fatty acids by *S. lactis* in its growth processes.

PEROXIDASE

Peroxidase oxidizes readily oxidizable substances by transferring oxygen to them from some peroxide, for example hydrogen peroxide. It can be demonstrated in milk or cream by adding to a small volume a few drops of hydrogen peroxide and some indicator which is readily oxidized and which gives a pronounced color when it is changed. Satisfactory indicators are freshly prepared 10 per cent gum guaiac in alcohol, 1.5 per cent aqueous solution of ortol, 2 per cent aqueous solution of paraphenylenediamine, etc.; a large number of indicators have been employed, some of which are mixtures of reagents. The indicator often is added before the hydrogen peroxide so that oxidases can be detected. Peroxidase seems to be more active in cream than in milk.

Rogers *et al.*[8] found that in cream pasteurized with the flash method peroxidase was absent after heating to 79 C (174.2 F), or above; at 77 C (170.6 F) it generally was absent; at 74 C (165.2 F), or below, it always was present. Spitzer and Taylor[44] reported that 62.5 C (144.5 F) for 20 min had no apparent effect on the peroxidase in milk, whereas flash pasteurization at 85 C (185 F) inactivated the greater part of it.

The peroxidase of milk affords a basis for determining whether milk and its various derivatives have been heated to a comparatively high temperature. This is important in enforcing certain pasteurization regulations, especially those dealing with pasteurization of factory by-products, but cannot be used to show whether market milk has been heated to the exposures usually employed because the temperature required to destroy peroxidase is too high. It is probable that in certain instances pasteurization requirements intended to prevent spread of animal diseases through skim milk, etc., were selected with the idea of the presence of peroxidase providing a means of detecting violations.

At one time a peroxidase test (Storch test) was widely used on butter in Canada where it is considered that, when cream has been pasteurized at 76.7 C (170 F) for 10 min, or longer, or at a higher temperature, peroxidase is destroyed. In making the test[45] about 2 oz of butter in a sample jar is melted in a water bath at 48.9 to 54.4 C (120 to 130 F). When the serum has separated about 4 to 5 ml are pipetted into a test tube. This is diluted with an equal volume of warm water. Two drops of hydrogen peroxide (1 vol of 3% peroxide and 2 vol distilled water) are added and then immediately 2 drops of paraphenylenediamine solution [2 gm dissolved in 100 ml distilled water·at 60 C (140 F)]. A decidedly blue color within 0.5 min indicates the sample contains peroxidase and the cream was not pasteurized; no color indicates the sample contains no peroxidase and the cream was pasteurized.

Peroxidase in butter sometimes seems to disappear on holding. Rogers *et al.*[8] found that in buttermilk it disappeared in about half the samples during holding for 1 yr and ascribed this to the slow digestion taking place. They stated that the peroxidase reaction in raw milk can be made to disappear in about 20 or 25 days by permitting the milk to sour, adding chloroform to eliminate subsequent bacterial action, and then allowing proteolysis by the lactic acid to go on, either with or without aid of pepsin; they also pointed out that salts of iron may be responsible for a reaction indistinguishable from a peroxidase reaction.

Dahle and Palmer[46] investigated the effect of storage on peroxidase activity of whole-milk powders and concluded that factors known to favor oxidation, such as air, heat, and moisture, proved detrimental to it. Powders stored in vacuum, or in containers which prevented entrance of air and moisture, showed greater peroxidase activity than the same powders exposed to air and moisture.

Elliott[47] obtained a crude but concentrated preparation of milk peroxidase, practically free of catalase, by fractional precipitation with ammonium sulfate. The activity decreased very slowly at 0 to 5 C (32 to 41 F). The enzyme was active from about *p*H 4 to nearly *p*H 10. From *p*H 8 to nearly *p*H 10 activity was diminished but recovered immediately on neutralizing. The enzyme oxidized nitrite quantitatively, as shown by usage of hydrogen peroxide or disappearance of nitrite. Later, Elliott[48] showed that very dilute hydrogen peroxide and peroxidase had no effect on various fatty acids, fats, sugars, most amino acids, and various metabolites. Other than the compounds used in testing for peroxidase, the only substances found to be oxidized were tryptophane, tyrosine, and nitrite. Peroxidase from horse-radish oxidized tyrosine but not the other two compounds.

In view of the limited oxidizing power of milk peroxidase it would be expected to have little influence on the deterioration of dairy products. Palmer and Miller[49] found that addition of peroxidase from horse-radish roots to pasteurized cream had no effect on keeping quality of the butter churned from it, either with or without ripening of the cream; some of the butter was stored at room temperature and some at 0 C (32 F).

Peroxidase may be of significance from the bacteriological standpoint. Demeter and Steude[50] reported that a substance hastening the killing of milk bacteria by heat was removed from milk by means of kaolin and 5 per cent ammonium chloride solution and found to be peroxidase. The substance gave the guaiacol reaction and was destroyed at 80 C (176 F). Added to peroxidase-free milk, it shortened the period required to kill bacteria at 62 C (143.6 F). Since bacteria of the coliform group destroy peroxidase, the accelerating effect may be absent from milk containing large numbers of these organisms.

CATALASE

Catalase splits hydrogen peroxide into water and molecular oxygen according to the reaction $2H_2O_2 = 2H_2O + O_2$. Its presence in milk can be shown by adding a small amount of hydrogen peroxide and observing the liberation of bubbles of oxygen. Use of a definite volume of milk with a standard amount of hydrogen peroxide and a tube in which the liberated gas can be collected and measured permits a comparison of the activity of the catalase in various lots of milk. Certain organisms produce catalase so that their action may be a factor in the results obtained, particularly when the test material contains many of them. For example, Sherman[51] reported that swiss-type cheese contains more catalase than cheddar cheese because of its characteristic bacterial flora.

Catalase is widely distributed in nature and is present in the milk of a variety of animals. Catalase contents of various lots of milk differ considerably, with colostrum and milk from cows advanced in lactation containing especially large amounts. Milk from abnormal udders often shows excessive amounts of catalase because of the presence of many leucocytes. Cream and separator slime contain more catalase than milk. Thatcher and Dahlberg[7] examined a number of samples of butter and noted that each contained catalase; storage did not appear to decrease its activity.

Rogers *et al.*[8] found catalase was destroyed in cream pasteurized by the flash method at 70 C (158 F).

Catalase apparently is of little importance in milk or its derivatives. Since excessive amounts sometimes are due to large numbers of bacteria or leucocytes or to some other objectionable condition, determination of the catalase activity of milk has been suggested as a test for the sanitary quality. Such a determination also has been proposed as a test for pasteurization; although catalase is not completely destroyed until rather high temperatures are reached, it is influenced at the temperatures commonly used in pasteurizing market milk.

PHOSPHATASE

Phosphatase hydrolyzes phosphoric acid esters with the liberation of inorganic phosphate and the alcohol. It regularly is present in milk and has been demonstrated in various ways, especially by determining the phenol liberated from disodium phenyl phosphate (Burgwald[52]).

Kay and Graham[53] noted that phosphatase is completely destroyed by efficient pasteurization, with either the holding or flash method, and that it is only relatively slowly destroyed by temperatures below 60 C (140 F) so that milk which has been heated for 30 min below 60 C still contains the enzyme. Later,[54] these investigators presented two methods of determining the efficiency of pasteurization that are based on the effect of heat on phosphatase. Extensive investigations have resulted in great improvements in the methods of determining phosphatase activity in milk. Procedures have been developed that distinguish accurately between properly pasteurized and inadequately heated milk; they detect slight irregularities in the temperature or period of heating and also contamination with a very small percentage of raw milk, at least as little as 0.1 per cent. Various phosphatase tests have been standardized[55] and they now constitute the accepted basis for determining whether pasteurization has been carried out satisfactorily. Some of the tests can be used in field studies.

Phosphatase is present in all raw milk. The amount varies with different animals and is influenced by the stage of lactation and other factors, for example, udder abnormalities. Wilson and Hart[56] found that the phosphatase activity of milk is usually higher than that of the blood plasma of the producing cow, and also that the activity in the milk is much greater in cows at the end of lactation than in heavy-producing cows at the beginning of lactation. In general, mixed herd milk varies little in phosphatase content.

The thermal stability of phosphatase is only slightly greater than that of the most resistant pathogens likely to be spread through milk. Over all temperature and time ranges, involving both holding and flash pasteurization, *M. tuberculosis* is destroyed more quickly than phosphatase, so that a pasteurization exposure sufficient to inactivate phosphatase, to the degree called for by the standards which have been developed, assures destruction of the pathogens normally spread through milk.

Phosphatase is not fat soluble but apparently tends to be absorbed in the protein layer of the fat globules. It is in the serum of butter. With various modifications, the phosphatase test can be applied to dairy products other than milk with satisfactory results.

The effect of various organisms on the phosphatase test is of special importance. Kay and Graham[53] pointed out that phenol-producing organisms may be present in milk and influence the results obtained with the phosphatase test. Gilcreas and Davis[57] reported that when pasteurized milk became sour it reacted as underpasteurized, but suggested that a considerable period of storage would probably be required to produce pasteurized milk which would give a typical reaction of raw milk; souring did not interfere with the determination of phenol. Paley[58] indicated that various bacterial cultures did not reactivate or increase the phosphatase content of milk to an extent which would interfere with the test; neither did old sour milk. Pasteurized milk held 5 hr at 37 C (98.6 F) did not show a reactivation of the enzyme. Leahy *et al.*[59] found with 28 cultures belonging to the genera *Lactobacillus, Streptococcus, Staphylococcus, Escherichia, Aerobacter, Salmonella, Klebsiella,* and *Pseudomonas,* that 1 strain of *Staphylococcus,* 5 strains of *Aerobacter,* and 3 strains of *Klebsiella* produced a true phosphatase reaction; additional strains produced a false phosphatase reaction. The authors suggested that phosphatase tests on milk having microscopic counts over 8 million per milliliter or plate counts over 2 million should be interpreted with caution.

Olson and Hammer[60] noted that various organisms produced phosphatase in sterile milk. Some of the species of the genus *Pseudomonas* were most active in this respect. These and other phosphatase-producing organisms also produced phosphatase in both salted and unsalted butter although production was less rapid in the salted product. An important point in connection with the formation of phosphatase in dairy products by bacteria is the ability of various *Pseudomonas* organisms to grow at relatively low temperatures.

Buck[61] reported a positive phosphatase test on properly pasteurized, commercial, bottled milk. The bacterial phosphatase responsible was produced by a thermophile which was named *Lactobacillus enzymothermophilus.* Production of the bacterial phosphatase in milk at 61.1 to 62.2 C (142 to 144 F) began after 90 min holding. Factors governing production of the phosphatase were size of inoculum, temperature, and holding period. The bacterial enzyme was destroyed at 85 C (185 F) for 1 min. Barber and Frazier[62] reported that lots of commercially pasteurized cream which originally were phosphatase negative became positive during 3 to 4 days at 4 to 10 C (39.2 to 50 F) and while still in a salable condition. The phosphatase-positive samples contained organisms that produced phosphatase; they were identified as *B. cereus* and *B. mesentericus.* When pure cultures of the organisms were inoculated into cream, the rapidity with which a positive phosphatase reaction developed depended on the numbers of organisms and amounts of bacterial phosphatase added by the inoculum. A positive reaction was obtained on pasteurized cream when the microscopic count of the organisms was between 1.48 and 7.8 million per milliliter. Bacterial phosphatase and milk phosphatase were active in the same general *p*H range, but in cream bacterial phosphatase withstood exposures as high as 76.7 C (170 F) for 30 min while milk phosphatase was inactivated at 62.8 C (145 F) for 30 min.

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Chapter 8

Spread of Diseases through Milk and Milk Derivatives

Spread of diseases through milk and milk derivatives constitutes the most important problem involving the bacteriology of these products. The prime consideration with any food material is its safety and when a product is used that occasionally contains pathogenic organisms its bacteriological condition is of special concern. Many of the present practices in the preparation and handling of dairy products are the result of attempts to prevent dissemination of pathogens through these products and the improvements continually being made are aimed primarily at increased safety.

Historical. The first important evidence of the spread of disease through milk was obtained by Dr. Michael Taylor[1] of Penrith, England, in 1857. This was before the science of bacteriology had been developed and before any organism had been proved the cause of a disease. Taylor observed a number of cases of typhoid fever that had only a milk supply in common. A servant girl had returned to her family in Penrith suffering from typhoid fever. She lived with her parents and four other children in a cottage consisting of two poorly ventilated and ill-lighted rooms. The father kept three cows and sold milk to about 14 families. The mother milked the cows and distributed the milk from the kitchen. Two of the other children in the family developed the disease and the mother nursed all three patients, while conducting the milk business. As soon as the servant girl was convalescent she helped with the distribution of the milk. Thirteen cases of typhoid occurred among seven families obtaining milk from the cottage, and from these primary cases a number of others developed. Taylor concluded that the milk was infected in the kitchen from the typhoid patients being nursed there.

Apparently, little immediate attention was given to the important possibilities suggested by the observations of Taylor, but this is not surprising when the extent of the bacteriological knowledge then available is considered. In 1867, an outbreak of scarlet fever occurred in Penrith and also was studied by Taylor.[1] Among a few scarlet fever cases there was a child that died after an illness of 2 or 3 weeks. In the cottage in which the child was nursed a small milk business was conducted. The milk was taken from the barn to the kitchen where it was strained and distributed to about 14 families. Fifteen cases of scarlet fever occurred in six of the families supplied and there also were secondary cases. Inquiries made by Taylor led to the conclusion that the disease was spread through milk as a result of its infection in the cottage.

Evidence that man may acquire infectious material from diseased cows through consumption of milk developed along somewhat different lines. In 1865, Villemin proved experimentally the infectious nature of tuberculosis by inoculating tuberculous material from man into rabbits and then soon afterward found that tuberculous material from cows also produced the disease in rabbits. The inoculability of tuberculosis and the relationship of the disease in man and cattle suggested its spread through milk and this later was proved by experiments in which various investigators produced the disease in animals by feeding milk from tuberculous cows.

The pioneer investigations showing that diseases can be spread through milk have been extended until hundreds of cases of various diseases have been traced to milk supplies. Such instances have been reported from all the important countries of the world and evidence showing the relationship of milk to the cases has been obtained by many different observers. With certain diseases, milk has been incriminated so frequently that it demands serious consideration in any attempt at their control, especially since the cases due to infected milk can be largely prevented through proper methods of production, processing, and handling.

Importance of dairy products other than milk in spreading diseases. Spread of diseases through milk suggests that products derived from milk also are of importance in this connection, although manufacturing methods may have a significant effect on the organisms involved. Ice cream frequently has been responsible for the spread of diseases and it is evident that its freezing and holding temperatures do not completely destroy any pathogens that may be present. In the ripened cheeses various disease-producing organisms apparently find it difficult to survive the conditions encountered, but a number of epidemics, some of them rather extensive, have been traced to the eating of relatively young cheddar cheese. Butter has caused only a few recorded outbreaks of diseases; this may be because butter retains only a comparatively small percentage of the organisms in the cream, but the destructive action of acid and/or salt on certain pathogens also is a factor.

The extended holding periods used with, and the wide distribution of, ripened cheeses and butter make it more difficult to trace cases of disease to these products than to milk or ice cream.

Spread of diseases among farm animals. Infected milk and milk derivatives also are of importance in the spread of diseases among farm animals and have been responsible for serious economic losses. Some of the early regulations intended to prevent the spread of pathogens through dairy products applied to milk returned to farms for animal feeding rather than to milk for human consumption.

Two groups of diseases spread through milk. Diseases that are spread through milk fall into two groups: Group 1, those in which the infectious material comes primarily from the producing animals; Group 2, those in which the infectious material comes primarily from human sources, either directly or by being carried in water, on utensils, etc. Group 1 includes diseases affecting both man and farm animals and one or more apparently affecting only farm animals, whereas Group 2 includes diseases that, for the most part, naturally affect only man. The diseases in the second group and certain of those in the first group often appear in epidemic form.

Decrease in spread of diseases through dairy products. Recognition of the importance of dairy products in the spread of diseases naturally led to various control measures, and at present milk and milk derivatives are not such common sources of pathogens as formerly. Important advances have been made in eliminating diseases from the producing herds. Pasteurization of milk and cream for direct consumption, and also for manufacturing purposes, is widely practiced. Attempts are made to prevent direct infection of dairy products from human sources by medical examinations of plant employees and by developing methods of handling pasteurized milk in plants so there is little direct contact between the product and the employees. Water supplies have been improved and returned containers are adequately treated to destroy pathogens that may be present. All these procedures have been significant factors in reducing the spread of diseases through dairy products and there now is little danger of contracting disease from adequately controlled supplies.

According to Armstrong and Parran [2] milk-borne epidemics in the United States reached a maximum in 1914 when there were 55 reported (238 for 1911–1915, inclusive). Since then there has been an encouraging decrease but the number still detected each year shows that much remains to be done in improving the sanitary quality of milk supplies, especially those in the smaller cities and towns where there may be little or no sanitary control.

GROUP 1. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES PRIMARILY FROM THE PRODUCING ANIMALS

TUBERCULOSIS

The danger in consuming milk from tuberculous cows was suggested by the results of a number of early investigators. In 1882, Koch[3] reported the discovery of the organism causing tuberculosis and then, after extensive studies, concluded that bovine and human tuberculosis are identical. However, the results of Smith, published in 1896[4] and 1898,[5] showed that there are important differences between the organism commonly causing the disease in man and the one responsible for the disease in cattle, and laid the foundation for a differentiation into the human and bovine types. The work of Smith at once raised the question of the importance of the bovine type of *Mycobacterium tuberculosis* as a cause of tuberculosis in man. At the British Congress on Tuberculosis in 1901, Ravenel presented evidence indicating that the bovine organism affects man and Koch reported that, on the basis of further experimental work, he no longer believed that the bovine organism was transmissible to man. The controversy thus begun led to extensive investigations in many countries and these show definitely that man contracts tuberculosis from diseased cattle.

Bovine organism in human tuberculosis. Because tuberculosis has been so largely eliminated from the dairy herds, the bovine organism now is of relatively little importance as a cause of human tuberculosis in the United States. At one time, however, it was of considerable importance. The work of Park and Krumwiede[6] in New York City, published in 1911, showed that the bovine organism was responsible for 1 or 0.33 per cent of 306 cases of tuberculosis in adults (over 16 yr); for 9 or 16.36 per cent of 55 cases in persons 5 to 16 yr; for 25 or 21.55 per cent of 116 cases in persons under 5 yr; and there was 1 double infection. If all the cases are considered, the bovine organism was responsible for 7.34 per cent. Park and Krumwiede[7] also tabulated data, including their own, obtained by investigators in different countries; some of the studies involved only a few cases and often these were selected because of the probability of the bovine organism being involved; so the percentages are not entirely representative. In the combined results (1,511 cases) the bovine organism was responsible for 15 or 1.57 per cent of 955 cases in adults; for 46 or 25.99 per cent of 177 cases in persons 5 to 16 yr; for 76 or 20.65 per cent of 368 cases in persons under 5 yr; and there were 11 double infections. Considering all the cases 9.13 per cent were caused by the bovine organism. The frequency with which the bovine organism caused tuberculosis in children is of special significance. Incidence of the bovine organism in human tuberculosis varies with the location of the lesions. From the data which they compiled, in 1912, when the bovine organism still caused considerable tuberculosis in man in the United States, Park and Krumwiede[7] prepared the following table illustrating this point:

PERCENTAGE INCIDENCE OF BOVINE TYPE OF *M. tuberculosis*
(Results of various investigators)

Diagnosis	Adults (Over 16 Years), Percentage	5 to 16 Years, Percentage	Under 5 Years, Percentage
Pulmonary tuberculosis	0.4	0.0	2.8
Tuberculous adenitis, cervical	2.7	38.0	61.0
Abdominal tuberculosis	20.0	53.0	58.0
Generalized tuberculosis, alimentary origin	14.0	57.0	47.0
Generalized tuberculosis	0.0	16.0	8.6
Generalized tuberculosis, including meninges, alimentary origin	0.0	0.0	66.0
Tuberculous meningitis	0.0	0.0	4.6
Tuberculosis of bones and joints	3.3	6.8	0.0
Tuberculosis of skin	23.0	60.0	0.0

Data from various countries, in which tuberculosis in dairy herds is not so completely controlled as in the United States, continue to emphasize the importance of the bovine organism as a cause of tuberculosis in man, especially as a cause of certain types of tuberculosis among children. For example, Price[8] studied 300 tuberculous children under 14 yr in Toronto and found that 15 per cent of the extra-pulmonary tuberculosis (for example, tuberculosis of bones, joints, lymph nodes, kidneys, and skin) was due to the bovine organism. The children infected with this type came to Toronto from widely scattered areas where there was no pasteurization and each child had consumed raw milk. The evidence definitely pointed to the alimentary route of infection. In three instances the bovine organism was demonstrated in the milk supply consumed by a child. A small outbreak of bovine infection occurred among children supplied by one dairy distributing raw pooled milk. No cases of bovine infection were detected in children from Toronto where pasteurization had been enforced for an extended period. The children infected with the human organism gave histories of contact with human cases.

Cases of human tuberculosis due to the bovine organism are more easily prevented than those due to the human organism because the former result largely from the consumption of infected dairy products, which can be rendered safe by suitable methods.

***M. tuberculosis* in milk.** Soon after *M. tuberculosis* was isolated the examination of milk to detect its presence began. In 1927, Tonney *et al.*[9] compiled data on 16,700 samples of milk that had been studied in various countries; 1,448 or 8.66 per cent were positive. In some of the more important cities in the United States during the period from 1907 to 1925 the percentages of the samples of milk found to contain *M. tuberculosis* varied from 2.7 to 44.0; most of them were in the range from 2.7 to 15.9. On the basis of their findings Tonney *et al.*[9] estimated that during the 3 yr prior to Jan. 1, 1926, approximately 43,750 qt of infected milk were sent each day to the plants pasteurizing milk for the Chicago market.

In many instances the detection of *M. tuberculosis* in milk has not been followed by a determination of the type. Tonney *et al.*,[9] however, studied the virulence for rabbits of the organisms obtained from their positive samples and found them all bovine.

Examination of milk for *M. tuberculosis*. Three methods have been suggested for the examination of milk for *M. tuberculosis:* (1) microscopic examination, (2) culturing under special conditions, and (3) animal inoculation.[10] Ordinarily, animal inoculation is the most satisfactory.

In examining a milk supply for *M. tuberculosis* one negative result means very little, because of the intermittent infection of the milk, and several examinations extending over a considerable period are advisable. Moreover, absence of the organism in a milk supply at one time means nothing as far as the future is concerned, and when tuberculosis is present in the producing animals the milk may suddenly contain the organism. If raw milk free from *M. tuberculosis* is desired, it can only be assured by keeping the cows free of the disease.

The methods used in examination of milk for *M. tuberculosis* are readily adaptable to the examination of other dairy products.

Methods by which milk from tuberculous cows is infected with *M. tuberculosis*. Infection of milk from tuberculous cows with *M. tuberculosis* is greatly influenced by location of the lesions and their general character.

When there is a lesion in the udder the milk commonly contains the causative organism. Briscoe and MacNeal[11] tabulated data reported in the literature and found that, of 39 samples from visibly diseased udders, 34 or 87.2 per cent were positive; most of the investigators reported results on only a few samples, owing no doubt to infrequency of the condition. In some of the studies on milk from individual cows the only samples found to contain *M. tuberculosis* were those from tuberculous udders. For example, Moore[12] noted in an examination of 87 samples of milk from individual animals that the 2 positive samples came from cows having udder infections. Milk from tuberculous udders often contains such large numbers of *M. tuberculosis* that many organisms can be seen in each field of a microscopic preparation made with sediment from the milk.

Some of the tuberculous cows without infected udders give milk containing *M. tuberculosis,* others do not. Briscoe and MacNeal[11] compiled data reported in the literature on the presence of the organism in the milk of tuberculous cows having no recognizable udder lesions and found that, of the 748 cows studied, the milk of 131 or 17.5 per cent was positive. Some of the samples were carefully drawn; with others there was the possibility of infection after the milk left the udder.

Additional ways in which *M. tuberculosis* can get into the milk of tuberculous animals are:

1. When the organism is present in the blood. Results obtained by the British Royal Commission[13] show that when *M. tuberculosis* is present in the blood stream it may appear in the milk without causing recognizable udder lesions. Inoculation of cows, either subcutaneously or intravenously, resulted in the presence of the organism in the milk and often it continued to be present for extended periods. In one instance a cow was inoculated subcutaneously with 100 mg of a culture of bovine origin and, after 155 days, which was the time of the last examination, the milk still produced tuberculosis in inoculated guinea pigs. The udder appeared normal and showed no lesions or *M. tuberculosis* organisms on microscopic examination. In 7 of 11 heifers that were inoculated subcutaneously with large doses of *M. tuberculosis,* the organism found its way into the milk sinuses of the undeveloped udders and, in at least 4 cases, was present in such numbers as to suggest multiplication there. While *M. tuberculosis* is not regularly present in the blood of tuberculous cows, it may enter the blood stream under certain conditions, the most common probably being when a lesion ruptures into a blood vessel. The spread of tuberculosis from one part of the body to another rather distant part, as well as the wide distribution of lesions sometimes noted, can best be explained on the basis of transmission of the organism through the blood stream. Lesions in various parts of the body following subcutaneous inoculation of experimental animals also suggest blood transmission, although other factors may be involved.

2. *When particles of infected manure fall into the milk.* Material coming from the lungs of tuberculous animals commonly is swallowed, thus infecting the fecal material. Tuberculous ulcers of the intestinal tract sometimes discharge enormous numbers of organisms and cause the same infection; these lesions appear to be rather uncommon in cows, however. The presence of *M. tuberculosis* in the feces of tuberculous cattle has been reported by various investigators.

3. *When material from an infected uterus gets into the milk.* With tuberculosis of the uterus, there is a possibility that organisms may be discharged and eventually get into the milk, perhaps most often by first contaminating the coat of the cow.

***M. tuberculosis* in dairy products other than milk.** With *M. tuberculosis* in milk, it also would be expected in such products as butter, cheeses, ice cream, etc., and the organism has been found in these products in various parts of the world. In the United States the infrequency of tuberculosis in dairy cows and the extensive use of pasteurization with commercial butter, cheeses, and ice cream make them of little importance as a source of *M. tuberculosis.*

Introduction of tuberculosis into a herd. There are two common methods by which tuberculosis is introduced into a herd: (1) through purchase of tuberculous animals, and (2) through return, for feeding purposes, of infected dairy plant by-products, such as skim milk, buttermilk, and whey.

The danger of introducing a tuberculous animal into a herd through purchase is so great that the tuberculin test now is widely used on animals bought, and a careful herdsman will not bring an animal into his herd until its freedom from the disease has been definitely established. Legislation recognizes the possibilities along this line and prevents some of the undesirable practices.

Return of infected dairy plant by-products has been an important factor in the spread of tuberculosis among farm animals. Russell[14] reported observations on the infection of cattle in this way. He noted that the percentage of animals reacting to tuberculin in the neighborhood of two Wisconsin creameries, the Medina and Oak Park creameries, was higher than the average, and also that the young stock raised on the different farms was infected, whereas often the mature animals, many of which were brought into the neighborhood, were not.

Of the 43 patrons of the Medina Creamery, 36 had their herds tested and 33 contained reactors, with 12 herds showing more than half the animals diseased. A total of 784 animals was tested and 271 reacted. Of the 3 herds not infected, the owners of 2 had moved into the locality within the year and brought all their animals with them. One farmer, who also had come into the section within the year, had 2 reactors in a herd of 28 but these had been bought from other herds. Another farmer, who had only 1 reactor among 48 animals, was a cream patron, and the reactor was the only animal in the herd that had been fed on skim milk from the creamery.

Twenty-four of the 36 patrons of the Oak Park creamery had their herds tested and 103 of the 429 animals reacted. The disease was found in 14 of the herds, but a high percentage of reactors was found in only 4. In 1 herd 24 of 26 animals reacted; in another 29 of 30 reacted. From the histories obtainable it appeared that practically all the reacting animals had been raised on the farms. Some time previously a herd tributary to the creamery had been destroyed because of heavy infection, 27 of 30 animals being slaughtered. This herd may have been an important factor in causing the high incidence in the neighborhood. Inquiry of the secretary of the creamery developed the fact that since the creamery was started the skim milk had been pasteurized by exhaust steam at 60 to 71.1 C (140 to 160 F) during the winter months, except during the winter the original heavily infected herd was destroyed, but that during the summer months no attempt at pasteurization had been made.

The percentages of infection in the tested animals in the Medina and Oak Park areas were 34.6 and 24.0, respectively. Among 1,467 animals tested in 78 herds whose owners patronized 11 neighboring creameries, 127 or 8.7 per cent reacted.

Information on the presence of *M. tuberculosis* in the milk of infected cows and in the various derivatives of such milk has resulted in extensive legislation requiring pasteurization of dairy plant by-products returned to the farms for animal feeding. These requirements have been important factors in limiting the spread of tuberculosis.

Financial losses due to tuberculosis in cattle. Although the primary interest in tuberculosis in cattle is due to its public health relationships, economic considerations also are involved. With tuberculous herds there may be financial losses due to occasional death of an animal, decreased milk production, exclusion of animals and milk from certain markets, and infection of hogs associated with them.

BRUCELLOSIS

Brucellosis in man is acquired primarily from animals infected with one of the organisms belonging to the genus *Brucella.* The species involved are *Brucella melitensis* (the caprine type), *Brucella abortus* (the bovine type), and *Brucella suis* (the porcine type). The three species are very closely related. Man is infected directly through contact with diseased animals or their tissues or discharges, or indirectly through consumption of raw milk and milk derivatives from certain diseased animals. The disease in man often is referred to as undulant fever. At one time it commonly was called Malta fever because of its importance on the island of Malta.

Jordan *et al.*[15] noted direct contact with hogs is a major factor in human brucellosis in areas producing many hogs. Direct-contact cases, whether from hogs or cattle, usually are sporadic and probably exceed in numbers the cases due to infected dairy products. Milk-borne brucellosis commonly is sporadic when *Br. abortus* is the etiologic agent. When *Br. suis* gains access to the udder of a cow and contaminates a raw milk supply, multiple human cases may be expected, presumably because *Br. suis* is more highly pathogenic than *Br. abortus* and multiplies more rapidly in a milk medium under ordinary atmospheric conditions.

Brucellosis from goats. Brucellosis has existed for an indefinite period on the island of Malta. The causative organism was discovered by Bruce,[16] in 1887, and named *Micrococcus melitensis,* in 1893.[17] Because of the prevalence of the fever among the British soldiers and sailors stationed on the island, the British government, in 1904, appointed a commission to investigate the sources of infection and advise methods of control. As early as 1905, the commission considered that milk from goats was an important factor in the spread of the disease among persons. Subsequent studies indicated that goats were easily infected, that about 50 per cent of them acquired the disease naturally, and that the causative organism was eliminated in the milk and urine. Ten per cent of the goats in Malta were found to be eliminating the organism in the milk, and this was highly infectious for monkeys, in which it produced attacks similar to the fever in man. It appears the elimination of the organism in the urine of goats is a late phenomenon and that this occurs in gushes for short intervals. Goats evidently are infected by consuming contaminated feed, and since the urine of infected goats may contain the organism there is abundant opportunity for this.

In the United States it was first established through agglutination tests and blood cultures that brucellosis existed endemically in the goat-raising sections of Texas. The human cases reported there gave a history of association with the goat industry, and some had consumed goats' milk. Many additional observations and tests show that the disease exists among goats in the United States.

Importance of goats' milk in spreading the disease has been shown in various ways. When the fresh milk supplied the soldiers at Malta was pasteurized, the disease among them was reduced 90 per cent. The disease did not occur among the convicts of the civil prison at Malta who were not allowed milk. It was practically eliminated among British soldiers stationed at Gibraltar by prohibiting the consumption of raw goats' milk. Mexican goat herders are only infrequently infected; this may be because they boil milk before drinking it.

History of a shipment of goats to the United States. The history of a shipment of goats to the United States[18] affords proof of the role played by milk from infected goats in causing brucellosis in man. A herd of 61 nannies and 4 billy goats was shipped from Malta, on the steamer *Joshua Nicholson* for the United States, via Antwerp. On reaching Antwerp the goats remained in quarantine 5 days and then went on the steamship *St. Andrew* to New York. The goats were transferred to a quarantine station where they remained under observation. Bacteriological examinations resulted in recovery of the *Brucella* organism, first from the milk of 2 goats and later from that of several more.

On the *Joshua Nicholson* there were 23 officers and men in addition to the persons in charge of the goats, and goats' milk was freely consumed. Eleven of the crew left the ship at Antwerp and, with the exception of one who was in the hospital because of a hernia, their movements could not be traced. Eight of the 12 remaining officers and crew fell sick at intervals varying from 18 to 34 days from the embarkation of the goats, and, in 5 of the 8, blood reactions showed that brucellosis was the cause of the illness. Of the 4 who were not sick, 2 drank very little milk and the other 2 appear to have boiled their supplies. The *St. Andrew* carried 30 cattlemen, a crew of 30, and the 4 persons in charge of the goats. Although many of these drank milk there was no evidence of illness. After the arrival of the goats in the United States a number of persons tasted the milk but only 2 drank any quantity. One of these doubted the idea of brucellosis being conveyed by goats' milk, and continued to drink the milk at intervals. He died suddenly from pneumonia and the agglutinating power of his blood against *Br. melitensis* never was tested. The other person developed a typical case of brucellosis.

Epidemic of brucellosis due to goats' milk. Lake [19] reported an epidemic of brucellosis that occurred in Phoenix, Ariz., as a result of consumption of goats' milk. More than 30 cases were positively diagnosed and undoubtedly many others occurred. In all but three of the cases goats' milk from the same dairy was known to have been used and in the three it could not be excluded. A *Brucella* organism was recovered from two of the cases. Agglutination tests were made on 115 goats in the herd responsible for the epidemic, and 18.3 per cent were positive.

Brucellosis from cattle. Contagious abortion in cattle has been known in England and Continental Europe for many years. In 1897, Bang [20] ascribed it to an organism which produced the disease on artificial inoculation and could be recovered from the infected animals. The organism now is designated *Br. abortus* and is the common cause of abortion in cattle. In 1918, Evans [21] noted the close relationship between *Br. abortus* and the organism originally described by Bruce as *M. melitensis.* This led to the conclusion that the latter organism is not a *Micrococcus* and suggested the possible relationship of *Br. abortus* to disease in man, a relationship which has been repeatedly confirmed.

***Brucella* organisms in milk.** Various investigators have observed that guinea pigs inoculated with cows' milk for the purpose of detecting *M. tuberculosis* and autopsied after extended periods showed lesions which could easily be mistaken for tuberculosis but in which no acid-fast organisms could be demonstrated. Schroeder and Cotton [22] noted this condition in a series of tests and found it transmissible from one guinea pig to another by subcutaneous inoculation of affected tissue. Their interest in the nature of the disease was continued when they found that the milk of a tuberculous cow caused it, even when the milk was obtained so as to exclude contamination from any source but the interior of the udder. The responsible organism was eventually cultivated and its similarity to the organism of contagious abortion led Mohler and Traum [23] to suspect that the two were identical; this suspicion was strengthened by the fact that much of the milk studied by

Schroeder and Cotton came from the sources that supplied Mohler and Traum with material for their work on contagious abortion. Comparison of the organisms showed no detectable difference, either cultural or morphologic, and use of the Schroeder and Cotton organism as an antigen in applying the complement fixation test to animals infected with abortion established the identity beyond a doubt. By inoculating the abortion organism Mohler and Traum produced typical lesions in guinea pigs.

Many studies show that milk from herds infected with *Br. abortus* frequently contains the organism. It can become established in the udder and multiply there and then appear in the milk, often in relatively large numbers. The udder presumably can be infected from the genital tract by discharges that contain the organism; transmission through the blood stream is another possibility. The percentage of samples of milk from infected cows that contain *Br. abortus* often is very high; Gwatkin [24] examined 102 such samples and found 52 positive.

Br. abortus also can get into milk from the stable surroundings. Results of Cameron [25] indicate it can survive for extended periods under a variety of conditions. It lived 4.5 hr exposed to direct sunlight, 121 days (maximum period studied) when dried in the presence of nutrient material, 72 days when dried in the absence of nutrient material, 66 days (maximum period studied) in wet soil in an unheated cellar, 4 days in normal bovine urine at room temperature, 120 days in bovine feces in a test tube in the laboratory and dried very slowly, and 100 days in bovine feces kept in an unheated cellar and not dried.

Dangers from infected milk. Cases of brucellosis in man occur each year in the United States and a portion of them are due to infected milk; but the number of cases is small when the wide distribution of the disease among cattle and the frequency with which infected cows eliminate the causative organism in the milk are considered. Hardy *et al.*[26] suggested that dairy products which serve to disseminate the infection carry either large numbers of organisms or strains of unusual virulence, and noted that relative human immunity also must be operative. Many human cases of brucellosis are isolated or in small groups, and it is evident that an extremely small percentage of persons consuming an infected milk supply develops the disease.

Information on the spread of brucellosis from cattle to man has emphasized the desirability of establishing herds that are free of the disease, and progress along this line has been rapid, especially in herds supplying milk that is to be consumed in a raw condition. The economic importance of brucellosis in cattle is another factor in the elimination program.

Examination of milk for *Brucella* organisms. Milk can be examined for *Brucella* organisms by culturing in tubes or on plates, using special procedures, or by animal inoculations.[10,27] Isolated cultures can be identified on a species basis. In some instances milk is examined for *Brucella* agglutinins because of their significance from the standpoint of the possible presence of *Brucella* organisms in the milk.

***Brucella* organisms in other dairy products.** With *Brucella* organisms in raw milk they would be expected in various milk derivatives, unless destroyed by heat, and they have been isolated on various occasions. When infected milk is separated, many of the organisms are carried into the cream.

Brucellosis from hogs. *Br. suis* apparently was first isolated in 1914 from premature fetuses from sows. The hog appears to be the true host. Like the other *Brucella* species, it is capable of causing disease in man. *Br. suis* apparently is more virulent for man than *Br. melitensis* or *Br. abortus.*

The primary source of human infection by *Br. suis* is contact with infected hogs or tissues from them on farms or in meat-packing plants. However, in some cases *Br. suis* becomes established in the udders of cows, and may cause disease in humans as a result of consumption of milk from the udders.

Beattie and Rice [28] studied an outbreak of milk-borne brucellosis that was caused by *Br. suis.* It involved 30 persons of whom 27 obtained milk from one dairy. This dairy had 20 cows and supplied about 80 households; in 18 of these, brucellosis occurred. The last case appeared 13 days after sale of milk from the dairy was stopped. *Br. suis* was obtained in blood cultures from 6 of 14 patients and from the milk of 1 of the cows in the herd.

A milk-borne outbreak of brucellosis due to *Br. suis* occurred in a home for elderly persons in Connecticut.[29] It involved 14 cases with 3 deaths out of a population of 305 inmates and 81 employees. The evidence indicated the disease was due to drinking raw milk from cows infected with *Br. suis.* The organism was isolated from the blood of 2 patients and an abscess in a third. Out of the herd of 37 animals the blood of 2 cows was positive for *Brucella* infection and that from another was suggestive. There was an opportunity for the infection of the cattle from a herd of infected swine.

FOOT-AND-MOUTH DISEASE

Foot-and-mouth disease is a highly infectious disease that affects various domestic and wild animals, especially cattle, and sometimes is transmitted to man. The virus is present in the fluid of the vesicles and, from this source, may gain entrance to saliva, feces, urine, and milk. Lesions sometimes occur on the udder; in certain instances the milk ducts and even the secreting portions of the udder are involved, and the milk then is highly infectious. There is some evidence that the milk does not contain the virus when the udder is free of lesions. Early in the disease the virus is present in the circulating blood.

Foot-and-mouth disease causes a reduction in the milk flow in cows and the milk produced may be very abnormal in appearance and flavor. Wing[30] reported that the daily milk production of an infected herd dropped from 4,000 to 1,200 lb in 6 days.

The disease in man may be contracted by contact with diseased animals, or through ingestion of infected milk, cream, butter, and cheese. In European countries, where foot-and-mouth disease has been common, many cases in man have been reported, sometimes in epidemic form. Children appear to be especially susceptible. The disease in man usually is mild, although occasionally the symptoms are severe and some cases end fatally.

Various outbreaks of foot-and-mouth disease have occurred in the United States, primarily among cattle, but these have been stamped out by quarantine and destruction of infected herds. Occasional cases of foot-and-mouth disease in man have occurred in the United States. Clough[31] reported one from Baltimore; proof of the nature of the disease could not be obtained through animal inoculation, but the clinical picture was characteristic. Infection may have come from milk or cream, since the disease was present in cattle at the time. Sutton and O'Donnell[32] reported a case from Kansas in which the diagnosis was based primarily on clinical evidence. In an outbreak in California, Wing[30] failed to find a case of foot-and-mouth disease in man under conditions which appeared favorable for its spread, since before the disease was recognized raw milk from infected farms was sold.

Milk or milk derivatives are a means of spreading foot-and-mouth disease from one farm to another. Wing[30] recorded an instance in which milk from an infected herd was separated and the raw skim milk shipped to another farm, where it caused foot-and-mouth disease in hogs to which it was fed; the disease then spread from the hogs to cattle. There was no connection other than the milk between the two farms, which were 57 miles apart.

JOHNE'S DISEASE

Spread of Johne's disease from one farm to another through dairy-plant by-products is suggested by the general nature of the condition. Characteristically, portions of the intestinal wall are thickened and contain large numbers of the responsible organism so that fecal material is readily infected. Hastings *et al.*[33] pointed out that desquamation of the epithelium probably is rapid in areas in which the organism is growing, and this suggests that shedding of the organism would be a steady, rather than an intermittent, process. Contamination of milk with infected fecal material would add the organism to the milk, and its transfer to other farms, either in milk, skim milk, or buttermilk, could spread the disease in the way that tuberculosis is spread. Feeding infected milk to calves on the farm where it is produced also may be a factor in extending the disease.

Lovell *et al.*[34] reported that, when exposed to atmospheric conditions, feces naturally infected with the organism causing Johne's disease may contain cells that are viable for as long as 246 days.

ANTHRAX

Bacillus anthracis may be present in the milk of cows that have anthrax. M'Fadyean[35] examined three samples of milk drawn post-mortem from cows that had died of anthrax, and in each anthrax bacilli were so numerous they were detected readily by microscopic examination. In an outbreak studied by Moore[36] the organisms were not found in the milk until just before, or soon after, death of the cows. About 14 animals died and for 3 weeks after the first case appeared and before the diagnosis was made the milk from the herd was retailed without injury to consumers. It was suggested that elimination of organisms with body discharges may afford an additional opportunity for the infection of milk. Morris[37] reported that *B. anthracis* remained virulent for 10 years in milk taken from the udder of a cow that died of the disease.

It is probable that the milk of cows having anthrax is not infected with *B. anthracis* until active multiplication of the organism in the blood stream begins. This usually occurs only shortly before death, and is accompanied by a temperature increase and other evidences of an abnormal condition, among which there ordinarily is suppression of milk secretion and change in its character. Accordingly, there is little danger from the milk of a cow having anthrax until the disease has progressed to a point where it is very evident. The general measures necessary to control the spread of anthrax from an infected herd make it inadvisable to ship the milk from the normal cows.

ACTINOMYCOSIS

The possibility of man acquiring actinomycosis through consumption of milk from infected cows is very slight. If the udder is involved the danger from *Actinomyces bovis,* as well as from secondary invaders that may be present in the lesions, presumably is greater than when the lesions are in other parts of the body. Cases of actinomycosis in man sometimes appear to be the result of infection through injuries to the mucous membranes of the intestinal tract, but the organism probably must be carried into the tissues.

Actinomycosis of the udder is uncommon in the United States. Kendall [38] reported a number of cases among the cows in Victoria that may be the result of an unusual prevalence of the causative organism on the pastures; the hind quarters were involved more frequently than the fore quarters. Albiston [39] also noted the frequency of clinical actinomycosis of the mammary glands of cows in Victoria. In the examination of 26 specimens, the causative organism regularly was found to be *Staphylococcus pyogenes.* Albiston concluded from a review of the literature and his own work that true actinomycosis (infection with streptothrices) of the udder is very rare. Examination of a small number of milk samples from cows showing clinical signs of mammary actinomycosis did not reveal any indication of infectious material from the nodules.

RABIES

Although cows occasionally develop rabies there is little danger of spread of the disease through milk. Peters [40] reported that on several occasions inoculation of rabbits and guinea pigs with milk from a rabid cow had given negative results. Nicolas [41] found that the milk and mammary tissue of herbivorous animals suffering from rabies never was virulent. Mugrage [42] noted that a small series of rabbits, either injected with milk from rabid does or nursing these does, did not develop rabies. He suggested that pasteurization, as ordinarily conducted, may not destroy rabies virus. Hardenbergh [43] stated that one authority had observed a doe that developed rabies while nursing her young; the little rabbits were kept for more than 1 year, and remained healthy.

TREMBLES AND MILK SICKNESS

Trembles is a disease of cattle and other farm animals. In certain cases the consumption of milk or milk derivatives from diseased animals causes a condition in man that is referred to as milk sickness. The disease in animals and man has been known for many years, at least since shortly after the middle of the eighteenth century, and was encountered by early settlers in many sections of the United States. Certain areas were regarded as milk-sick areas and were carefully avoided, in some instances by fencing. As the country was settled the disease gradually decreased until now it has disappeared in areas where it once was common. Wolf *et al.*[44] stated, in 1918, that the disease probably occurs in the mountainous portions of all the counties of western North Carolina and in the immediately adjacent sections of states having similar conditions. In 1926, Marsh *et al.*[45] reported that a disease of cattle, commonly called alkali poisoning or milk sickness, had long been known in the Pecos Valley of New Mexico and Texas, and that a disease in man was identified with it.

The theories as to the cause of trembles and milk sickness have been along three main lines: (1) mineral poisons, (2) micro-organisms, and (3) poisonous plants. Certain plants now are generally regarded as responsible.

Plants responsible for the disease. Poisonous plants were considered to be the cause of the disease by some of the early observers. This theory was supported by various facts, such as period of the year the disease is most common, and its disappearance as the land is cultivated. Although a number of plants has been accused, white snakeroot or richweed (*Eupatorium urticaefolium*) has been blamed most often. In 1917, results of various investigations were reported which definitely established that one plant which causes the disease is white snakeroot. Curtis and Wolf[46] developed 15 cases in sheep by feeding the plant, 14 of which resulted fatally; death occurred in 5 to 27 days following beginning of the feeding. Marsh and Clawson[47] found that white snakeroot was toxic for both cattle and sheep. Moseley[48] noted that an ether extract of the leaves of white snakeroot, when consumed by rabbits, affected them similarly to the leaves themselves. In 1918, Wolf *et al.*[44] reported an extended series of experiments in which they produced disease in various animals by feeding white snakeroot. The investigations were carried out where no case of the disease had ever appeared prior to the trials, and the white snakeroot was shipped 400 miles. During the studies, 31 fatal cases developed among 44 ewes and lambs used in various phases of the work, 1 of the 2 hogs employed developed typical trembles, and 15 of 29 guinea pigs died from eating white snakeroot or its products. Two lambs contracted the disease by suckling their mothers, proving that the disease can be transmitted through milk. It was not possible to determine with certainty whether death resulted from the feeding of butter to mice. Animals appeared to differ greatly in their susceptibility to poisoning with white snakeroot. Sackett[49] reported feeding experiments with white snakeroot and various extracts of it, which indicated that guinea pigs are more resistant to poisoning than rabbits. The viscera and flesh of rabbits that had died from the poisoning were not harmful to a cat.

Results of Marsh *et al.*,[45] who studied the milk sickness encountered in the Pecos Valley, showed that typical symptoms could be produced in cattle, horses, and sheep by feeding sufficient quantities of rayless goldenrod, or jimmy weed (*Aplopappus heterophyllus*). It was found that the toxic substance could be excreted in the milk, and that calves and lambs may be poisoned in this way; the findings suggest that cases in man may occur from consumption of milk or butter from cows feeding on the rayless goldenrod.

Toxic constituent of white snakeroot and rayless goldenrod. Investigations of Couch[50] indicate that tremetol, $C_{16}H_{22}O_3$, is the constituent of white snakeroot that is responsible for milk sickness. The compound has the properties of an unsaturated alcohol, and is soluble in fat and fat solvents; it may be secreted in milk and, because of its solubility, would be carried into butter. A resin acid and a volatile oil also are present in white snakeroot but are incapable of producing disease. When white snakeroot is dried the tremetol content rapidly decreases and the completely dried plant is not toxic. The tremetol content of white snakeroot from different sections varies widely, and in some areas the plant apparently is not dangerous.

Later, Couch[51] reported that tremetol also is the toxic constituent of rayless goldenrod, and that the dry plant appears to lose its toxicity slowly, the process being much less rapid than with white snakeroot.

Distribution of cases of milk sickness. Couch[52] pointed out that milk sickness is primarily a rural disease. Outbreaks have occurred principally on farms and in small towns where persons often consume milk from one or a few cows. In cities, the milk comes from many sources, and diluting milk from cows having trembles with normal milk ordinarily renders it harmless.

Couch [52] noted that tremetol is only slowly destroyed by the temperature of boiling water so that pasteurization probably affects it only very slightly.

MASTITIS

The relationship of organisms to mastitis was early recognized as a result of the development of an infection when materials containing organisms were injected into the udder through the teat canal, and by appearance of an inflammation when a normal udder was injected with material from one that was diseased.

General condition. Variations occur in the extent and type of udder infections because of differences in resistance of various animals and in virulence of the causative organisms. In severe mastitis the affected quarter may be intensely inflamed, feverish, and indurated, whereas in mild cases the abnormality may be very slight and difficult to detect. Often only one quarter is abnormal but in certain cases two or even more quarters are involved. With a severe infection the milk may be abnormal in appearance; with a slight infection it may not. Many infected udders quickly return to a condition that appears normal, although the causative organism may persist for extended periods. Occasionally, one or more quarters are so severely involved that milk secretion ceases and that portion of the udder is lost for the time being, or even for succeeding lactation periods also.

Organisms causing mastitis. Among the organisms causing mastitis, streptococci occur most frequently, staphylococci are next in importance, and then come various organisms that are only occasionally responsible.

The species most often responsible for mastitis is *S. agalactiae* (*Streptococcus mastitidis*). It is a typical bovine type that is not pathogenic for man. Attempts to infect persons with massive doses of this organism have been unsuccessful; also, milk containing it frequently is fed to farm animals without causing disease. Bovine types next in importance as a cause of mastitis are *Streptococcus dysgalactiae* and *Streptococcus uberis.* Among the staphylococci, *Staphylococcus aureus* appears to be involved most often but other species occasionally are encountered. Additional organisms that infrequently cause mastitis are coliform organisms, with *A. aerogenes* [53] most important but with intermediate types and *E. coli* sometimes involved, *Corynebacterium pyogenes, Pseudomonas aeruginosa,* etc.

Rarely, mastitis is caused by typical human streptococci that gain entrance to the udder directly or indirectly from some person. The organisms sometimes find favorable conditions in the udder and multiply extensively. As a result the freshly drawn milk contains enormous numbers of bacteria that are pathogenic for man and extensive epidemics have been caused in this way. The disease in man most often caused by the human types of organisms coming from the udder is septic sore throat, but there are other possibilities, especially scarlet fever.

In some cases organisms growing in the udder produce toxic substances that cause poisoning when the milk is consumed. Staphylococci appear to be most often responsible.

Organisms causing mastitis frequently are spread from one animal to another in a herd by the hands of the milkers, by milking machines, and in various other ways. Mastitis thus presents a problem in herd management. Because of the economic losses involved, as well as public health considerations, attempts frequently are made to free the herds of mastitis and maintain them in this condition.

GROUP 2. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES DIRECTLY OR INDIRECTLY FROM MAN

In the United States the most frequent milk-borne diseases belonging to this second group in which the infectious material comes from man, either directly or indirectly, are typhoid fever and septic sore throat, and there are others of minor significance. Although the organisms responsible for septic sore throat commonly come from the udders of the producing animals, they are primarily human types and the udders evidently are infected from human sources. When milk-borne, the diseases in this group often occur in epidemic form in the same manner as when they are spread in other ways. However, milk-borne epidemics have certain characteristics which are helpful in deciding whether the epidemic under investigation is due to milk.

Major characteristics of milk-borne epidemics. The more definite characteristics of milk-borne epidemics and those of most help in detecting infected milk supplies are the following:

1. Cases are largely confined to users of milk from one source. If an epidemic is due to milk from a certain source, the cases are largely confined to persons consuming it, whereas epidemics from other sources, such as water, do not follow a given milk supply. Accordingly, when a considerable percentage of the cases in an epidemic are along the route or routes of one dealer, a milk-borne outbreak is indicated. However, if a dealer furnishes a large part of the milk sold in a community, consumers of the supply may have a high percentage of the cases in an epidemic in which milk is not involved; for example, if a dealer sells 95 per cent of the milk, many cases would occur among the consumers in an epidemic due to city water or some such cause. In general, with the usual complexity in milk distribution, a considerable number of cases among consumers of a given milk supply and not among consumers of other supplies is suggestive.

Various factors tend to limit infections among persons consuming milk from an incriminated supply, some of which are:

(*a*) Not all portions of a supply causing an epidemic contain the responsible organism and, undoubtedly, some households escape in an epidemic because the milk coming to them happens to be free of the pathogens.

(*b*) In certain homes milk commonly is heated, often to boiling, before it is consumed, and with sufficient heating the pathogens present are destroyed. When the milk is used entirely for cooking or baking there ordinarily is no danger from it.

(*c*) Persons vary in their resistance to different diseases and it is probable that in most milk-borne epidemics certain individuals escape through their immunity. The increasing use of immunization procedures is of importance in this connection.

The percentage of the persons using an infected supply that contract the disease varies greatly, owing to the above factors. In some instances it is very low and in others it may be nearly 100 per cent. Undoubtedly, infections have occurred through drinking only one glass of milk.

2. *Epidemics often are explosive in nature.* With milk-borne epidemics of any considerable magnitude, a number of persons acquire the pathogens at about the same time and, with a fairly definite incubation period, this results in a number of cases appearing simultaneously. In some instances a given supply is infected over a considerable period and cases then continue to appear; in others the milk is infected only once or twice and all the cases are reported in a comparatively short period. Two or even more cases may develop in a family on the same day.

In addition to epidemics due to milk, those spread through general water or food supplies also may be explosive in nature. Accordingly, the simultaneous appearance of a number of cases of a disease that may be milk-borne suggests a general source of infection, with milk one of the possibilities.

3. *Most cases are in homes consuming considerable milk.* If an epidemic is milk-borne it would be expected that with a fairly uniform distribution of the pathogens throughout the milk supply, homes con-

suming large quantities of milk would show more cases than those using little milk, provided the milk was not treated by a method which destroyed the causative organism. In the past, families with the smaller incomes often have materially limited their milk consumption, so that in some milk-borne epidemics cases have been more numerous in families having moderate or large incomes. With the present agitation for increased use of dairy products, milk consumption is rather heavy in many families having comparatively small incomes.

4. Most cases are among heavy milk drinkers. Heavy consumers of milk have a greater chance of being infected during a milk-borne epidemic than light consumers and, proportionately, more cases occur among them. Children consume much milk and would be expected to show a high case incidence; but the milk they drink often is heated in the homes so that they may show a low rate of infection.

Minor characteristics of milk-borne epidemics. In addition to the characteristics listed, a number of others that are more or less unusual have been noted in certain milk-borne epidemics. The incubation period sometimes has been rather short for epidemics in which the date of infection of the milk was established. The causes suggested are heavy infections, due to growth of the organisms in the milk, and rapid absorption of the organisms. Certain epidemics have shown a low mortality but with others the mortality has been normal, or even high. Milk-borne epidemics have been more common in medium-size and small towns than in cities; the reason is that much raw milk is consumed in the small communities and, accordingly, less of the milk is subjected to a process that makes it safe. Some of the milk-borne epidemics that have occurred in cities have been very extensive, owing to the wide distribution of the milk.

In milk-borne outbreaks of various diseases there may be a different age distribution of cases from that in outbreaks spread in other ways. A relatively high incidence in children has been noted in certain milk-borne outbreaks of typhoid. Godfrey [54] considers that the milk supply should be investigated whenever an unusual number of adult cases of scarlet fever or diphtheria occur in a community.

Methods of tracing milk-borne epidemics. Methods of tracing milk-borne epidemics are epidemiological rather than bacteriological. Isolation of the pathogens from a suspected supply normally is not attempted for the following reasons: (1) Satisfactory methods are not available and the failure to find the organism involved in an epidemic is no assurance that it was not in the milk; (2) considerable time is required to examine milk for one of the pathogens and in an epidemic a decision as to the source of the causative organism must be made quickly so that necessary control measures can be instituted; (3) the organism responsible for a milk-borne epidemic is not continuously present in the supply, and, since the cases must have been infected some time before they began to appear (because of the incubation period), a supply may have caused an epidemic and still not contain the responsible organism at the time an examination is attempted.

The usual procedure is to obtain data on each case, either directly from the individual or from someone else who is thoroughly informed, and then analyze them in an attempt to locate some possible source of the organism that is common to all, or the great majority, of the cases. If practically all the cases in an epidemic have used a certain milk supply and have had nothing else in common, the milk is at once under serious suspicion. The data obtained may point to some source such as water, foods of various sorts, contact with certain diseased individuals, etc., in quite the same way that milk is indicated. A spot map illustrating the distribution of the cases along the different milk routes is valuable when milk is a possible source of the infection, since this shows quickly and in a striking manner any grouping of cases along a particular route. The point to be considered especially is whether or not a certain milk route shows an excess of cases over the number to be expected. If a given route has 80 per cent of the cases but at the same time is delivering about 80 per cent of the milk in the locality, it has the percentage that would be expected if the source was not milk, and it does not have an excess of cases. However, 80 per cent of the cases occurring on a route supplying only a small percentage of the milk is at once suspicious, if nothing else common to the cases is detected.

Care is necessary in obtaining data on the cases since some incident that is not readily recalled may be important in tracing the epidemic. A single glass of milk from a supply other than the usual one may be the only connection between a given case and the milk causing the epidemic, and if this is recorded it furnishes valuable confirmatory evidence when the source of the epidemic is not clear.

Tracing an epidemic is essentially an elimination process in which one possibility after another is excluded until the evidence finally points to one remaining source. The possibilities to be considered in an investigation depend on the particular disease involved; for example, the common sources of diphtheria epidemics are quite different from those of typhoid epidemics. Water supplies so carefully controlled that they are known to be safe ordinarily make the detection of milk-borne typhoid much easier than when uncertain water supplies must be considered. In reporting to health departments cases of diseases that can be spread through milk, there is an advantage in including the sources of the milk supplies since a tabulation of such data may show at once a case distribution indicating a milk-borne epidemic and quickly lead to recognition of an infected supply.

If the findings incriminate a milk supply, valuable confirmatory evidence is obtained through location of the source of the organism, for example, a carrier, a definite case, an infected water supply, etc. When a suspected supply is stopped, or pasteurized if it is raw, and the outbreak subsides, the exact time depending on the incubation period of the disease in question, further proof is provided.

TYPHOID FEVER

Numerous epidemics of typhoid fever in various countries have been traced to milk and its derivatives, and undoubtedly many others due to these products have been included with those epidemics that have not been solved. Milk has been involved most frequently, with ice cream next in importance. Butter-borne typhoid has been rare. Recently, there have been a number of outbreaks of cheese-borne typhoid. In general, they have been due to young cheddar cheese; as a result of them, there now are regulations in certain areas requiring pasteurization of milk for cheese unless the cheese is held for a definite period, the assumption being that this period is so long that any typhoid organisms originally in the cheese would have died. These regulations are in agreement with regulations in many areas covering the pasteurization of milk and cream for various purposes.

Example of milk-borne typhoid. An extensive milk-borne typhoid epidemic occurred in Montreal.[55] From March 1 to July 16 there were 5,014 cases with 488 deaths. Two peaks occurred in the epidemic, one about the middle of March and the other about the middle of May. Water supplies were excluded because the distribution of cases was unrelated to the two separate and distinct supplies, and also because Verdun, an adjoining city with a separate water supply, had many cases. Milk was accepted as the cause of the outbreak because of the age distribution of the cases, 903 being under 10 years and 718 between 10 and 20 years, and because a large percentage of the cases used the products of dairy M.D.C. In institutions, such as boarding houses, orphanages, and homes for helpless adults, there was a high case incidence when milk from dairy M.D.C. was used but a normal incidence when the milk came from other dairies. The supply distributed by dairy M.D.C. came from 1,200 to 1,500 farms and it was estimated that this milk was exposed more or less to a population of 20,000. Some of it came through four receiving stations, one of which used water from a polluted river. At the city plant the milk was pasteurized in a machine of an efficient type, but as it was operated there appeared to be some leakage from the pasteurizer before pasteurization was complete. The plant foreman was found to be a carrier and was relieved of duty on April 16. Accordingly, he could not have been the cause of the cases during the second peak of the epidemic. It appeared that a portion of the raw milk coming to the plant was distributed without being pasteurized, since at certain times more raw milk was delivered than was run through the pasteurizing machine.

Example of butter-borne typhoid. A typhoid epidemic occurring at Anoka, Minn.,[56] eventually was traced to butter. Cases of typhoid were developing and a suspected milk supply had been exonerated. The population included numerous persons previously ill with typhoid. After search for the main route of infection had eliminated public water supplies, wells, flies, milk, and contact, it was noted that most of the cases occurred in one ward and all patronized one grocer so that a food infection appeared probable. Eventually, a farm was located from which butter was sold to the grocer during the illness and convalescence of an unreported case of walking typhoid in a hired girl. At the time of the report between 35 and 40 cases with 6 deaths had occurred.

Example of cheese-borne typhoid. In a cheese-borne outbreak of typhoid that occurred in Alberta,[57] 83 cases were reported with 7 deaths. Some of the cases were the result of having eaten infected cheese and others were contacts of the primary cases. The outbreak extended over an area about 100 by 300 miles and involved several cities as well as isolated areas. Investigation of the epidemic revealed that all the early cases had eaten fresh, green cheddar cheese which for the most part had been purchased at the same market. The cheese had been manufactured in a small factory from raw milk brought from near-by farms. At the factory about 2 weeks usually elapsed between manufacture and grading of the cheese, after which it moved directly to the retailer. Examination of samples of the cheese about 48 and 63 days old for *Eberthella typhosa* gave only negative results. A survey showed that milk came to the cheese factory from 96 farms; 223 persons handled the milk in one way or another and of these 19 gave histories of having had typhoid and 51 of contact with typhoid. A carrier was detected in the first group. This woman had had the disease in Russia, in 1919, and all three stool samples were positive. *E. typhosa* cultures from 6 cases traceable to the infected cheese and from the carrier were of the same type.

Records of the Alberta Health Department showed that between Jan. 1, 1936, and Sept. 30, 1944, 507 cases of typhoid occurred in the province, of which 111 were directly traceable to consumption of infected cheddar cheese; the three cheese-borne epidemics occurred in 1936, 1938, and 1944.

Sources of *E. typhosa* in milk. The sources from which *E. typhosa* gets into milk vary widely. Importance of the various sources has changed with the more widespread use of pasteurization and the improvement in plant methods.

In 1927, Armstrong and Parran[2] summarized the sources of the organism in 373 outbreaks, for which the sources were known, as follows:

Sources from Which Milk Was Infected with Typhoid Organisms

Probable Source of Infection	Number of Outbreaks
Carrier (farm, distributing plant, etc.)	162
Active case (farm, distributing plant, etc.)	134
Exchange of bottles from homes with infection	37
Use of polluted water on utensils, etc.	28
Soilage of cows in polluted water	4
Miscellaneous (intermediate persons, etc.)	8

The results indicate the importance of carriers and active cases.

In modern milk plants pasteurization largely controls the dangers from carriers and cases on farms, more adequate bottle washing and sterilizing equipment reduces the danger from returned bottles, and better water supplies largely eliminate the chances of typhoid organisms getting into milk from water. Carriers and cases in the plants still are a hazard although modern milk-handling procedures, which greatly reduce contact of persons with the milk, minimize it.

PARATYPHOID FEVER

The spread of paratyphoid fever through dairy products shows the same general features as the spread of typhoid. Although only a comparatively few epidemics have been reported, the number is large enough to indicate the possibilities along this line.

Example of milk-borne paratyphoid. An outbreak of paratyphoid affecting primarily infants and young children occurred in New

Rochelle, N. Y., and 9 neighboring communities.[58] A milker at a dairy selling certified milk who was found to be a carrier of paratyphoid B was the source of infection.

The investigation included 50 case histories, information being obtained from either the family physician or the parents. The youngest patient was 3 months old, and 49 of the 50 patients were under 6 years; the remaining patient, 40 years of age, was on a special diet that included certified milk from the dairy.

Each of the patients used the milk in question, and there were no similar cases at the time among persons using any other milk. Water was excluded because the area involved was served by a number of separate supplies. The clue to the diagnosis of the outbreak was the isolation of paratyphoid B from the stool of one of the children during convalescence. The organism then was obtained from 3 other children. Paratyphoid B was isolated from the stool of the carrier, and also from the stool of his 4-year-old child. The last raw certified milk from the dairy was sold on May 7, and the last case reported became ill on May 13.

DYSENTERY AND DIARRHEA

The general characters and methods of distribution of the various organisms responsible for dysentery and diarrhea suggest that these diseases can be spread through dairy products and a number of epidemics have been traced to them.

SEPTIC SORE THROAT

Although outbreaks of milk-borne septic sore throat have been recognized in Europe for many years, comparatively little attention was given them in the United States until 1912, when several serious epidemics occurred. Milk-borne septic sore throat is included in the group of diseases in which the infectious material comes primarily from man but often the milk appears to have been infected from abnormal udders; in these cases, however, the types of organisms suggest that the udders were infected from human sources.

Septic sore throat is the one disease in which epidemics are very largely milk borne. Although many cases are due to contact, definite epidemics commonly include cases with more or less characteristic symptoms and quite regularly involve milk supplies. The outbreaks frequently are extensive and, accordingly, attract a great deal of attention; a number of those that have occurred in the United States have involved more than 1,000 cases each and an outbreak in Chicago was estimated to include more than 10,000. The mortality is low, with the deaths occurring principally among old people as a result of complications.

Examples of milk-borne septic sore throat. Ex. 1. An extensive epidemic of sore throat occurred in Chicago. Capps and Miller[59] studied its relationship to the milk supply, and Davis[60] investigated the causative organism. More than 10,000 cases were involved.

Physicians attending the cases agreed that the epidemic was quite different from ordinary tonsillitis. After milk was suggested as a cause, investigation showed that most of the cases were consumers of milk from dairy X. Of 173 cases selected because of their severity and conformity to the epidemic type of throat infection, 147 (85%) were users of milk from dairy X at the time of illness; 15 (79%) of the 19 fatal cases used milk from this source. In the districts investigated, dairy X supplied about 10 or 12 per cent of the milk. Of the households using milk from dairy X, 51 per cent were affected as contrasted with 7.2 per cent of those using other milk. The average morbidity for affected households using milk from dairy X was 2.3 persons; for affected households supplied with other milk it was 1.2 persons. Accordingly, the ratio of morbidity among users of milk from dairy X to morbidity among users of other milk was about 14 to 1. Incidence of the disease was studied among the nurses in hospitals; the total number of nurses using milk from dairy X was 153 and of these 80 (52%) were attacked, whereas the total number using milk from other dairies was 721 and of these 35 (4.8%) were attacked. At one hospital the 70 occupants of the children's wards passed through the epidemic without a case of sore throat, but among the nurses and internes there were many cases. All the milk for the hospital came from dairy X but the milk for the children was delivered raw and pasteurized at the hospital; the remainder of the milk came from the usual pasteurized supply. At Batavia, Ill., which was the center of the dairy X herds and where the milk was pasteurized, there was a great deal of sore throat, not only among customers of dairy X but also among customers of other dairies.

Sore throats of the epidemic type were present on 11 farms supplying dairy X, with a total of 29 cases. On 9 of these farms there also was mastitis among the cows. One cow had a chronic abscess in a hind quarter, and although milk from this was thrown away that from the other quarters was used; a girl on the same farm was very ill with sore throat. On another farm milk from a cow having typical mastitis of long duration contained streptococci, and streptococci were also obtained from a girl who had been ill with sore throat 5 weeks before.

Both human and bovine cultures were fatal to guinea pigs and rabbits. On a third farm all three milkers had sore throats and there were 11 cases of mastitis among the cows. The milk sold by dairy X was flash pasteurized, supposedly at a temperature of 71.1 C (160 F). Fluctuations in the temperature occurred, however, with a minimum of 54.4 C (130 F) on several days. There were pronounced failures of the pasteurization on Dec. 17, 19, 28, 30 and on Jan. 7 and 11, and explosive outbreaks of cases appeared to follow these dates rather closely. Final responsibility for the epidemic undoubtedly rested on inadequate pasteurization.

Ex. 2. In April, 1945, Bowering *et al.*[61] investigated an epidemic of septic sore throat affecting 136 persons in Salmon Arm, B. C., a town having a population of about 900. The outbreak was confined to the town, with only very few cases in the surrounding rural district. It was explosive in nature, lasted 1 mo, and involved almost as many adults as children. Of the persons infected, 81 per cent obtained their milk from the same dairy. Investigation revealed that an employee of the dairy had been ill with a severe sore throat some time previous to the epidemic; that one of the cows developed mastitis subsequent to this, following an udder injury; and that all persons having to do with the handling of the milk on the farm were among those involved in the epidemic.

Example of septic sore throat due to reconstituted milk powder. Allen and Baer[62] reported an outbreak of septic sore throat due to reconstituted milk powder that apparently was contaminated in a mechanical cow at a naval station. About 10 per cent of the complement of the station was ill. An inspection of the galley and food handlers was made early in the epidemic. Defects noted in the dairy room and preliminary case records made it seem probable that milk was the source of the infection. A rapid subsidence of the outbreak followed discontinuance of its use.

The epidemiological evidence incriminated the general mess because 100 per cent of the patients had eaten at the mess, whereas large groups of personnel at the station eating at other messes were not sick. Of the first 100 patients admitted to the sick list, 100 gave a history of drinking milk. No other food could be similarly incriminated. One of the two men assigned to work in the milk preparation room gave a history of having had a sore throat 2 weeks before the outbreak and he had a recurrence during the epidemic. The study was considered to indicate a new source of danger to military personnel.

Sources of causative organisms in milk-borne septic sore throat. The probable sources of the causative organisms in 42 milk-borne septic sore throat epidemics were summarized by Armstrong and Parran;[2] cases on farms were involved in 16 epidemics, mastitis in cows in 3, cases on farms and mastitis in cows in 9, carriers on farms in 2, and the source was undetermined in 12. The more recent outbreaks also emphasize the importance of cases, carriers, and mastitis in cows as sources of the organisms involved.

Ramsey[63] reported data on 13 epidemics of septic sore throat, due to raw milk, that were investigated in detail at the time of occurrence or shortly after. In 7 instances human illness had occurred on the incriminated farm prior to the outbreak; in 4 of these there were sore throats and in 3 draining wounds or hand infections in dairy workers. Human cases occurred on the suspected farm in 4 epidemics while they were in progress but not previously, as far as could be determined. In only 2 epidemics was it impossible to find evidence of illness among persons on the farms involved. Ramsey interpreted these results as indicating that when septic sore throat occurs the original source of the infection is more likely to be an ill person than a healthy carrier.

SCARLET FEVER

Although scarlet fever is contracted principally through direct contact, the causative organism occasionally gets into dairy products and then may reach susceptible persons.

Examples of milk-borne scarlet fever. Ex. 1. Welch and Mickle[64] reported an outbreak of scarlet fever and septic sore throat that epidemiological evidence indicated was due to heavy infection of a milk supply. Of 5,000 quarts delivered in the area, 240 came from the dairy involved; 68 of the 100 cases were in families using the infected milk, and most of the others were clearly accounted for by contact infections. Of 70 families residing in the part of town where the outbreak occurred, 32 used the infected supply and 18 had 44 cases of scarlet fever and septic sore throat. The 14 families taking infected milk but having no cases included 4 that reported scarlet fever previously, 7 that consisted of 14 adults and 1 child, and 3 that scalded their milk. The organisms isolated from clinical cases of scarlet fever and sore throat showed the biochemical and morphologic characteristics of *Streptococcus scarlatinae*. Two milk handlers operating the dairy connected with the outbreak on epidemiological evidence harbored in their throats organisms identical in every respect with those isolated from clinical cases.

Ex. 2. An epidemic of approximately 500 cases of scarlet fever was traced to use of raw milk from an infected cow.[65] The source of the outbreak was suspected early in the epidemic and sale of raw milk from the dairy concerned was stopped; this action was followed by rapid decline in the number of cases. Investigation revealed that the suspected animal had been taken into the herd at the dairy farm a few days previous to the beginning of the outbreak. Immediately prior to that, the cow had been on a farm where three cases of scarlet fever had occurred, one in a boy who had milked the cow; the evidence indicated the animal had acquired the infection there.

DIPHTHERIA

Diphtheria is contracted principally through direct contact with human sources, either cases or carriers but, occasionally, eliminated organisms encounter conditions that permit their survival for rather extended periods and a number of epidemics have been traced to dairy products.

Example of milk-borne diphtheria. Hedrich [66] reported an instance in which diphtheria appeared to be spread through an infection on the outsides of milk bottles. About 15 positive throat swabs were received in a city laboratory, 1 or 2 a day over a period of 10 days. Nearly all the patients were under 6 yr of age, which suggested a milk-borne epidemic. Investigation showed that most of the involved families patronized one milk dealer who served about 1,500 customers with pasteurized milk through five wagons. The small number of sporadic cases among a large number of customers was soon explained. Among the milk handlers there was a person whose throat contained the diphtheria organism. Part of his work was to inspect bottles for cracks and he easily could have infected his hand and then the outsides of the bottles when he lifted them by the neck. The diphtheria organism presumably got into the milk when it was poured from the bottles. The epidemic was stopped by quarantining of the carrier.

POLIOMYELITIS

A disease rather recently added to the group that is sometimes spread through milk is poliomyelitis. The evidence indicates that it is only rarely contracted in this way.

Examples of milk-borne poliomyelitis. Ex. 1. Aycock [67] reported an epidemic of poliomyelitis that occurred in Broadstairs, England, during October, 1926. The outbreak started and subsided suddenly, 62 of the 75 cases being reported between Oct. 14 and 29; 31 of them were reported on Oct. 4 and the 5 days following. Multiple cases occurred in private boarding schools having little communication with the town or with each other. In the 5 yr previous to the outbreak only 2 cases of poliomyelitis had been reported in Broadstairs.

Practically all the cases were supplied with milk from dealer A. Although three boarding schools in which cases occurred were supplied by dealer F, these may have obtained milk indirectly from dealer A, since from Sept. 23 to Oct. 15 he supplied 44 quarts daily to dealer F. Four persons who left Broadstairs shortly before the occurrence of the epidemic developed the disease, and while in Broadstairs each of them had lived in a house supplied by dealer A.

Of the total 75 cases, 50 were known to have used milk from dealer A, and 19 others (in private boarding schools) probably received this milk. All the 43 paralyzed cases could be associated with milk from dealer A, 35 directly and 8 through dealer F. Dealer A supplied different grades of milk, including grade A which came from a tuberculin-tested herd, and although the data were incomplete there was a high proportion of grade A users among the cases. This suggests that the milk was infected on the farm producing the grade A product.

Ex. 2. An explosive outbreak [68] of poliomyelitis at a naval training school involved 18 diagnosed cases with a possibility of at least 100 milder infections. Data accumulated during an epidemiologic investigation suggested food, probably milk contaminated by flies, as the source of infection. Milk used was mostly raw, frequently had high bacterial counts, and regularly contained flies; the flies in the area had access to human and animal feces. Poliomyelitis virus was isolated from the feces of 3 of 4 patients tested.

CHOLERA

A number of milk-borne epidemics of cholera have been recorded and such outbreaks are to be expected from the frequent distribution of the disease through water. In certain countries in which cholera is common the practice of boiling milk probably prevents many infections. *Vibrio comma* apparently is easily destroyed by acid developed in milk or cream.

Examples of milk-borne cholera. During World War I the British Army operating in the Near East experienced acute epidemics of cholera in two hospitals [69] between Aug. 24 and 31, 1916. In one hospital there were 35 cases with 14 deaths, and in the other 19 cases with 10 deaths. In both instances the infection was carried by the milk supply. The milk commonly was sterilized before it was given to the patients, but it often arrived late and the sterilization was not always supervised.

ADDITIONAL MILK-BORNE DISEASES

From time to time an additional disease is found to be milk borne. This can be illustrated as follows:

Murphy *et al.*[70] reported that on the basis of circumstantial evidence an outbreak of infectious hepatitis was traced to a single milk supply. The sequence of events and distribution of cases followed the general pattern observed in milk-borne outbreaks of other diseases. Prior to the outbreak at least 2 cases of the disease lived in close proximity to the source of the milk supply. Conditions at the dairy were such that contamination of the milk was possible. Within the limits of the incubation period, cases appeared among consumers of the milk but not among non-consumers, who were twenty times as numerous.

INTESTINAL DISTURBANCES IN CHILDREN

Intestinal disturbances in children may be responsible for illnesses and deaths, especially among young children. These conditions appear to be related, in part, to the food, since various data show that cases have been much more common among artificially fed infants than among those that are breast fed, although they are by no means confined to the former group.

Much of the early work on milk improvement was stimulated by the many deaths among bottle-fed infants and better milk supplies have been a factor in the decrease of such deaths. Often excessive numbers of organisms of various types in milk appear to have been related to the illnesses and deaths, rather than specific types, but in certain instances definite species presumably were involved. The attention now devoted to the feeding of infants from the standpoint of the modification of the milk and its heat treatment has largely eliminated the bacteriological condition of milk as a factor in intestinal disturbances, except where carelessness prevails.

POISONING FROM DAIRY PRODUCTS

Various dairy products are included in the large group of food materials that may cause poisoning. In some instances dairy products have been considered responsible for poisoning on very meager evidence, but in other cases careful investigations have completely established their relationship to the condition.

A number of ways in which dairy products may acquire poisonous properties have been suggested; these include (1) conditions involving the producing animal, such as elimination of drugs, substances contained in the feed, and substances resulting from disease in the animal, and (2) conditions developing after production of the milk, such as absorption of metals and action of micro-organisms. Of the conditions involving the producing animal, elimination of poisonous products contained in the feed is the most important and is illustrated by milk sickness. Of the conditions developing after production of the milk, action of micro-organisms, either directly or through products elaborated by them, is the most important.

The observations of Burke *et al.*[71] are of interest in connection with absorption of metals. After deaths among swine that were attributed to buttermilk held in a galvanized iron tank, the investigators carried out studies that showed buttermilk dissolved zinc from surfaces of galvanized vessels in proportion to the acidity and period of exposure. Under the conditions of the experiment, souring of milk in galvanized containers had no deleterious effect on growth and reproduction in rats and swine. Small amounts of zinc lactate had no marked effect on growth and reproduction in rats and swine, but a relatively large amount of zinc carbonate (1% or more) appeared to retard growth and reproduction in rats.

Examples of poisoning from dairy products. Ex. 1. Macaulay[72] reported an outbreak of cheese poisoning that occurred in Dover, England. The cheese involved was of the cheddar type and came from Canada; because of its rather distinctive appearance, the cases investigated could be definitely traced to it. Out of a total of 135 individuals who were known to have eaten the cheese, 126 included in 43 families were attacked. In every case the symptoms began a short time after a meal at which the cheese was eaten, and no other article of food was common to all the cases; the incubation periods varied from 3.5 to 11 hr. Three persons, after recovery from the initial attack and not suspecting the cheese, ate it a second time and experienced another attack. The symptoms regularly included abdominal pain, while diarrhea, vomiting, and prostration were common. There were no deaths. The cheese appeared perfectly wholesome. A few small holes, apparently due to gas, were noted in pieces of it and did not appear in other cheese of the same lot. Feeding trials with mice gave irregular results, but in some instances symptoms of poisoning were noted. A rabbit that was fed twice with large doses of the cheese remained unaffected. The one trial with a human subject yielded definite poisoning. No organism known to be associated with food poisoning was found in the cheese or in specimens from cases, and mineral poisons could not be detected. The outbreak was considered to be almost certainly due to a bacterial toxin.

Ex. 2. Homemade cottage cheese, which had caused the death of three persons who had eaten it, was studied by Nevin and Mann.[73] An anaerobic organism was isolated that killed mice, guinea pigs, and rabbits, and these species also were killed by filtrates of cultures; the minimum fatal dose of toxin, 0.0005 ml of filtrate of a 72-hr culture, killed guinea pigs in 4 days. By repeated injections of heated toxin, an antitoxin was obtained from a rabbit which protected a guinea pig against 100 times the minimum lethal dose of toxin. The organism was identified as *Clostridium botulinum.* Inoculation of 5 ml of the toxin into a kitten gave the typical picture of botulism.

Ex. 3. Chason and Waite[74] investigated an outbreak of gastroenteritis involving 11 persons, in 4 families, who became acutely ill with vomiting and diarrhea and concluded it was due to ingestion of homemade buttermilk. From the buttermilk a culture of *Staph. aureus* was isolated which, under proper conditions, produced an enterotoxin demonstrable by the Dolman kitten test. The source of the milk implicated in the outbreak was a family cow that showed no physical evidence of mastitis and her milk did not yield growth of the causative organism. Examination of the milker and of the wife of the vendor gave no evidence of sores on the hands, etc. There was no sterilization of the utensils in which the milk was collected, stored, or sold.

Ex. 4. Weed *et al.*[75] presented data indicating that 3 patients developed food intoxication after drinking milk of a goat suffering from acute suppurative mastitis due to *Staph. aureus.* Two of the patients (3 and 4 yr of age) died within 24 hr after each drank one-fourth pint of the milk. Cooked milk did not cause illness. When the milk was drawn, nothing unusual was noted except that the yield was lower than normal. The goat died within 24 hr. The only significant findings post-mortem were limited to the udder, one side of which was greatly swollen, red, and firm. An incision into the swollen side revealed marked congestion with numerous dilated lactiferous ducts containing a light reddish-brown fluid suggestive of bloody milk. No definite grossly visible abscesses could be found. Material from the udder post-mortem reproduced the symptoms in kittens. There was a history of a scratch on a teat 2 weeks earlier.

Ex. 5. Tucker *et al.*[76] studied an outbreak of food infection involving 250 cases in Tennessee, 100 cases in Illinois, and 34 cases in Kentucky. All the patients had eaten cheese (colby) 24 to 48 hr prior to the onset of illness. In many families persons became ill after eating cheese and persons not eating cheese remained well. The cheese was on the market soon after manufacture. Bacteriological examination of a sample of cheese resulted in isolation of *Salmonella typhimurium.* At 6.1 to 8.9 C (43 to 48 F) the organism remained viable in a 6-lb piece of the cheese for 302 days, examinations being made at monthly intervals.

Identification of responsible organisms. In studies on food intoxications and infections, the isolation and identification of the responsible organisms frequently are attempted, the methods that are used being determined by the general nature of the illness and other considerations. To obtain proof that an organism which appears to be responsible for an outbreak actually was involved often is difficult and very time consuming.

Because so many outbreaks are due to staphylococci, various attempts have been made to develop rapid methods of identifying enterotoxin-producing strains. Haynes and Hucker [77] concluded that such strains are more frequently pigmented, produce acid from lactose and maltose, and grow on alkaline media in the presence of bromthymol blue. The active enterotoxin-producing types generally liquefied gelatin and hemolyzed washed red cells of rabbits. Ability to produce coagulase correlated to a high degree with enterotoxin production. The authors noted that presence of large numbers of orange or, less commonly, white hemolytic, coagulase-producing types of the organisms in a suspected food is presumptive evidence that such organisms may have caused the epidemic. Feldman [78] concluded that those staphylococcal strains that produce coagulase, hemolysin, and golden pigment were more likely to produce enterotoxin but this combination of characters did not occur with sufficient regularity to replace the cat test.

GROWTH AND LONGEVITY OF PATHOGENS IN DAIRY PRODUCTS

Milk. Although the nutrients provided by milk are adequate for the growth of various pathogens, multiplication under practical conditions is influenced by such factors as holding temperature, reaction, and other species present. Brown [79] found that *E. typhosa,* paratyphoid, the dysentery organism (Shiga), and *C. diphtheriae* grew in milk but that growth was less rapid at 21.7 to 25.6 C (71 to 78 F) than at 36 C (96.8 F). Exposure to low temperatures, such as 5.6 to 6.7 C

(42.1 to 44.1 F), caused no appreciable destruction of bacteria during the first 48 hr and there may even have been some multiplication by continued exposure, but exposure for 3 weeks caused rapid destruction of the individual bacteria most susceptible to low temperatures.

Wahby and Sherman[80] studied the growth of pathogens in milk, using three species of the typhoid-dysentery group, four of the paratyphoid group, *Br. abortus, C. diphtheriae, V. comma, S. epidemicus, S. scarlatinae,* and *S. agalactiae.* The trials were conducted in skim milk that had been sterilized so that counts of the added organisms could be made more accurately than if the milk had contained its original flora. At 30 C (86 F) all the organisms grew rapidly; at 15 C (59 F) all of them grew except *Br. abortus* and *V. comma,* although the growth of *C. diphtheriae* and one of the dysentery organisms (Shiga type) was feeble; at 10 C (50 F), the four paratyphoid species grew and *E. typhosa* sometimes showed very slight growth after several days, while the other organisms failed to grow; at 7.5 C (45.5 F) and at 5 C (41 F) none of the organisms grew.

Pullinger and Kemp[81] found that various species of *Salmonella* multiplied readily in fresh raw milk at 15 C (59 F) and also in commercial grades of raw and heat-treated milk.

Although *M. tuberculosis* develops well on sterile milk held at favorable temperatures, multiplication probably never occurs in milk under practical conditions because the organism has a rather definite growth temperature, especially when it has just come from a lesion, and also because growth starts slowly.

Various investigations show that as lactic acid is developed in milk *E. typhosa* is gradually killed. Krumwiede and Noble[82] found the rate of destruction in sour cream to be proportional to the degree of acidity and the number of typhoid organisms. They noted that with moderate contamination the typhoid bacilli are killed in about 4 days, whereas with heavy contamination, or where initial multiplication has taken place, a longer period may be required. Heinemann[83] used a sterilized mixture of milk and lactic acid and found the limit of acid tolerance of *E. typhosa* to lie between 0.3 and 0.4 per cent; the organism was destroyed at about 0.42 per cent and there was a great reduction at 0.35 per cent. The investigator noted that acid-tolerant strains apparently multiply in amounts of acid destroying the majority of cells. He also suggested the possibility of bacterial products other than acid playing a part in destruction of such organisms as *E. typhosa.* Wilson and Tanner[84] found that *E. typhosa,* two *Salmonella* species, and two *Shigella* species were destroyed more rapidly when lactic acid was added to milk than when natural fermentation occurred and that slow increases in acid permit development of acid-tolerant species if they are present. The authors noted that in naturally infected milk the acidity developed is not sufficient to destroy, in a reasonable period, the pathogens tested; with 0.95 to 1.15 per cent acid (*p*H 4.2 to 3.9) the organisms survived 1 day to 7 weeks and with 0.50 to 0.86 per cent (*p*H 5.0 to 4.3) they persisted 2 to 9 weeks.

Ice cream. In ice cream stored in a hardening room that fluctuated from −22.2 to −13.3 C (−8 to 8 F), Prucha and Brannon [85] found that there was a continual decrease in the number of inoculated typhoid organisms but that some still were alive after 2 yr and 4 mo, when the examinations were terminated. Wallace [86] found various pathogens, including *Salmonella, Brucella,* and *Mycobacterium* types, survived in ice cream for several years.

Butter. The viability of pathogens in butter was investigated by Berry; [87] the butter was stored mainly at ice-box temperature, which usually was 10.5 C (50.9 F), although a few samples were held at room temperature. After inoculation of the butter there was an immediate decline in the numbers of organisms in most cases. The organisms studied showed wide variations in their viability in butter. Paratyphoid B survived 212 days. Strains of *E. typhosa* varied considerably; one strain survived only 22 days or less, another survived 63 days, and still another survived at least 110 days. Paratyphoid A survived 117 days, the dysentery organisms disappeared in 7 to 18 days, and *S. scarlatinae* survived a maximum of 17 days. Berry found that in milk at ice-box temperature paratyphoid B survived 324 days, paratyphoid A 107 days, a strain of *E. typhosa* 290 days, and *S. scarlatinae* 17 days. Washburn [88] noted that in an ice chest *E. typhosa* lived 151 days in butter and 43 days in milk; it was actively motile in milk for 20 days. In connection with the study of a butter-borne diphtheria outbreak, the Minnesota State Board of Health [89] found the organism in butter after 1 mo.

Bryan and Bryan [90] infected a cow with two streptococci and two staphylococci, each in a separate quarter of the udder, and after collecting the cream added a broth culture of *Br. abortus* and made butter from it. At about 7.2 C (45 F) *Br. abortus* remained viable 4 mo in raw sweet cream butter and 3 mo in raw ripened cream butter. *S. agalactiae, S. pyogenes,* and both hemolytic and non-hemolytic *Staph. aureus* persisted in raw sweet cream or raw ripened cream butter for 6 mo. Results were the same with 2 per cent salt in the butter as with no salt.

Butter made from the cream after pasteurization gave only negative results.

Early studies on the period that *M. tuberculosis* remains alive in butter gave variable results but suggested that the organism persists for considerable periods. More recent data involving modern storage conditions indicate clearly that the organism will live as long as butter ordinarily is stored. Moore [91] reported a trial in which infected butter still produced the disease in guinea pigs after 97 days in an ice box. Mohler *et al.*[92] found that salted butter stored at either −23.3 C (−10 F) or −12.2 C (10 F) produced tuberculosis in some of the inoculated guinea pigs after 6 mo but not after 9 mo; one lot of unsalted butter stored at −12.2 C gave essentially the same result. Tuberculosis also was produced in guinea pigs by feeding the butter but not as regularly as when it was injected. The milk from which three lots of the butter were made was artificially infected, but milk from a tuberculous udder was used to inoculate the milk for one lot of salted butter and the lot of unsalted butter. The investigators called attention to the uneven distribution of *M. tuberculosis* in butter, which was shown by samples failing to infect guinea pigs at one examination and later proving positive. Schroeder and Cotton [93] examined salted butter made with milk from a tuberculous udder and found that it contained *M. tuberculosis* after 160 days when held at 15.6 C (60 F). Briscoe [94] reported that *M. tuberculosis* was alive in artificially infected butter stored 274 days at −10 C (14 F), 4 C (39.2 F), or approximately 20 C (68 F); the disease in the inoculated animals was most severe with the butter held at the lowest temperature.

Cheese. Wade and Shere [95] reported a cheese-borne typhoid epidemic in which the development of cases indicated *E. typhosa* lived in the cheese approximately 63 days; the cheese was made from milk which did not curdle satisfactorily. In 18 experimental cheese made from milk infected with *E. typhosa,* the organism usually lived from 0 to 8 days, averaging about 4 days, but with two cheese the organism was recovered on every test until no more material was available, which was on the thirty-fourth and thirty-sixth days after manufacture. Since these two cheese were made from the same lot of milk, longevity of the organisms in the cheese may have been influenced by the flora of the cheese. In trials involving inoculation of *E. typhosa* into milk to which acid had been added, it appeared that the degree and character of the acid affected survival of the organisms.

Campbell and Gibbard [96] studied survival of *E. typhosa* in small commercial-sized cheddar cheese. The data show there was a significant difference in the viability in cheese stored at 4.4 to 5.6 C (40 to 42 F) and at 14.4 to 15.6 C (58 to 60 F); both lots of cheese were held at 14.4 to 15.6 C for 14 days before being waxed for holding. In the cheese held at 14.4 to 15.6 C most of the *E. typhosa* cells were dead after 3 mo, whereas the cheese held at 4.4 to 5.6 C contained viable *E. typhosa* cells for at least 6 mo and in 7 out of 10 cases for 10 or more months. There appeared to be no difference in the longevity of the organisms when small or large inocula were used, but a difference in resistance of different types of the organism was suggested.

Marquardt and Hucker[97] found that *S. agalactiae* was present in cheese made from low curd tension milk after curing 9 mo.

Gilman *et al.*[98] studied the survival of *Br. abortus* in cheese. Cheddar cheese was made from milk produced by a herd highly infected with brucellosis. The milk contained about 500 viable *Br. abortus* organisms per milliliter and a freshly isolated culture was added to make about 1,000. The organism survived in the cheese for 6 mo at 4.4 C but only negative results were obtained after 1 yr. With milk produced by cows eliminating large numbers of *Br. abortus,* there were 700 to 800 of the organisms per milliliter. All the cheese was positive after 3 mo, some were negative after 6 mo, and all were negative after 1 yr. Two vats of limburger cheese milk in New York were positive to *Br. abortus* but the curds were negative and the cheese was negative when first examined after 57 days. With 34 vats of commercial cheddar cheese milk in New York and Wisconsin, 9 lots of milk and 8 lots of curd were positive to *Br. abortus.* Two cheese were lost and the other seven from the positive lots of milk were all free from viable *Br. abortus* when first examined after 41 to 84 days at 1.1 to 2.7 C (34 to 36.9 F).

Harrison,[99] working at Berne, investigated the survival of *M. tuberculosis* in experimental cheeses of the swiss and cheddar types, the organism being added to the milk; in swiss cheese it died in 33 to 40 days, in cheddar cheese it lived for at least 104 days. He repeated in Canada the work on the period the organism will survive in cheddar cheese and found it died in 60 to 62 days. Mohler *et al.*[100] reported that infected cheddar cheese obtained by adding the organism to the milk produced generalized tuberculosis in guinea pigs into which it was inoculated when it was 220 days old, and that slight lesions were produced after 261 days. Feeding also caused the disease in guinea pigs but not so frequently as inoculations. Fifty samples of swiss cheese made of milk from tuberculous cows were examined by Kankoanpaa;[101]

7 (14%) contained *M. tuberculosis.* The organism was found in naturally infected cheese 200 days old, but the number was low or the virulence weakened. In artificially infected cheese of different types, *M. tuberculosis* lived for varying periods, the maximum being 113 days.

General considerations. Although results of different investigations on the survival of a particular pathogen in a dairy product sometimes vary considerably, the general data indicate that pathogens may live in dairy products for extended periods. Under these conditions attempts to ensure the safety of such a product as raw-milk cheese by holding for a given interval is open to question. This is especially true when a low holding temperature appears to extend the period an organism will survive in such a product as cheese and requirements covering the holding normally ignore the holding temperature.

The apparent increase in the number of cheese-borne outbreaks of typhoid during recent years has emphasized the danger of using raw milk for manufacturing purposes. The logical solution of the problem is pasteurization of the milk for cheese; this is in agreement with the widespread use of heat treatment with other dairy products.

LABORATORY EXAMINATIONS OF DAIRY PLANT EMPLOYEES

The importance of human cases and carriers of various diseases as sources of pathogens in dairy products suggests the examination of dairy plant employees in an attempt to locate persons who may infect supplies of such products. Frequent physical examinations are of value in this connection but laboratory tests provide a more adequate means of locating potential sources of pathogens. The diseases for which examinations should be made are suggested by the frequency with which various diseases are spread through milk and its derivatives and by the probability of detecting cases and carriers of these diseases.

Certain organizations require examination of employees coming in contact with dairy products. In some cases, at least, the examination is of such a type that it gives only very incomplete and inadequate information. Moreover, to control the possibilities reasonably well, examinations should be made at rather frequent intervals.

In some instances employees permitted to work in contact with dairy products have sore throats or other conditions that may be spread through the products. The dangers from such individuals obviously are greatest when they come in contact with a product following its pasteurization.

SUMMARY OF MEANS OF CONTAMINATION OF DAIRY PRODUCTS WITH PATHOGENS

Consideration of the spread of diseases through dairy products shows that the responsible organisms gain entrance to these products from a variety of sources. They can be summarized as follows:

1. Animals producing the milk.
    - *a.* Interior of the udder.
    - *b.* Ulcers, etc., on teats and udder.
    - *c.* Coat of the animal, feces, etc.
2. Persons in contact with milk and its derivatives in stables, in dairy plants, on routes, etc.; including true cases, carriers, and individuals associating with persons that are ill.
    - *a.* Direct infection of dairy products.
    - *b.* Infection of utensils, equipment, etc.
3. Infected water.
    - *a.* Used for washing utensils, equipment, etc.
    - *b.* Splashing into milk or cream when used for cooling, etc.
4. Air and dust of stables, dairy plants, etc.
5. Flies and miscellaneous sources.

Although there are sources other than those listed from which pathogens may get into dairy products, they represent unusual instances and are of little significance. The sources outlined cover a period from before the milk leaves the udder until it, or the product made from it, is consumed, and with these possibilities the incidence of diseases spread through dairy products is not surprising.

There is great variation in the dangers from the different sources; for example, the possibility of addition of pathogens to milk from the air of the milk room is very slight, whereas there have been many epidemics due to infected persons coming in contact with milk. The dangers from any one of the sources vary with the different diseases. Addition of *M. tuberculosis* to milk in the interior of the udder often has occurred, whereas *E. typhosa* probably never has been added to milk in this manner.

One of the important factors involved in the relationship of the various sources to the different diseases is the methods by which the causative organisms leave the body of the responsible person or animal. The possibility of the survival of the organisms after leaving the body also is a factor. Infected water is of importance in connection with the spread of typhoid through milk because elimination of *E. typhosa* through the excreta results in occasional infection of the water that is used in washing dairy utensils. Air of the milk room causes the infection of milk only rarely because desiccation and light rapidly destroy the pathogens that most frequently get into it.

PREVENTING DISSEMINATION OF DISEASES THROUGH DAIRY PRODUCTS

The methods of preventing spread of diseases through dairy products are (1) prevention of entrance of pathogens, and (2) destruction of any pathogens that may be present.

Consideration of the sources from which pathogens get into dairy products shows that the thorough control of all of them is a tremendous undertaking that begins with the health of the producing animals and ends only when the dairy product is used. Perhaps the greatest problem is in connection with the persons coming in contact with dairy products, since any one of them may be normal one day and capable of eliminating pathogens the next. Attempts that are made to control all the sources of pathogens in milk add greatly to the cost and such specially produced milk is not available in the great majority of cities and towns. Moreover, some of the attempts to prevent the entrance of pathogens into milk have not been successful and cases of disease have resulted. Epidemics of various diseases have been spread through milk produced under conditions that were intended to be excellent. On the other hand, careful supervision undoubtedly has prevented epidemics.

Destruction of the pathogens that may be present in milk and cream is most readily carried out by proper pasteurization, and this controls the dangers up to the time of the treatment. There is the possibility of infection of milk and cream after pasteurization, but this is small compared to the possibility of infection before the milk and cream reach the pasteurization plant and can be controlled by proper equipment and methods. The pasteurization must be efficient if the anticipated protection is to be obtained; a number of epidemics have been traced to milk that was improperly heated because of some defect in the equipment or its operation. Proper pasteurization provides an inexpensive method of controlling the spread of diseases through dairy products and, because of the low cost, is applicable to the general supply of these materials. Improvement in the keeping qualities of milk and cream also results from pasteurization and the saving derived from this is considerable when the products are exposed to severe conditions.

Accomplishments of milk control. Where well-organized attempts have been made to control milk supplies, a great deal has been accomplished. This is illustrated by the following data reported by Feemster [102] on milk-borne diseases in Massachusetts; presumably, during the early part of the period covered, recognition of milk-borne outbreaks was very incomplete.

DECREASE IN MILK-BORNE OUTBREAKS OF DISEASE IN MASSACHUSETTS

Period	No. Outbreaks	Period	No. Outbreaks
1886–1890	3	1916–1920	32
1891–1895	4	1921–1925	17
1896–1900	15	1926–1930	14
1901–1905	20	1931–1935	7
1906–1910	28	1936–1940	4
1911–1915	45	1941–1945	3

The results show that since the 1911–1915 period there has been a striking decrease in the number of milk-borne outbreaks in Massachusetts.

Data on the milk-borne disease outbreaks in the United States are compiled each year by the U. S. Public Health Service from reports submitted by state and local health officials; a summary of the results is as follows:

TOTAL REPORTED OUTBREAKS OF MILK-BORNE DISEASES IN THE UNITED STATES

Year	No. Outbreaks	Year	No. Outbreaks
1923	23	1934	42
1924	44	1935	43
1925	44	1936	42
1926	68	1937	43
1927	36	1938	42
1928	46	1939	41
1929	51	1940	43
1930	48	1941	37
1931	34	1942	45
1932	33	1943	40
1933	42	1944	41

The data show that in the United States as a whole a relatively large number of outbreaks of milk-borne diseases still occur each year. Undoubtedly, recognition of milk-borne outbreaks has been more complete recently than it was formerly but it has been reasonably complete for a rather extended period. Many of the outbreaks occur in areas where there is only inadequate milk control. Frequently, a raw milk supply is involved but pasteurized milk also is included because of inadequate heat treatment of a part or all of the milk or contamination of properly pasteurized milk. The most significant changes in the types of outbreaks occurring have been a decrease in outbreaks of typhoid and an increase in outbreaks of food poisoning and gastroenteritis.

The general situation indicates the need for greater emphasis on the control of milk-borne outbreaks in certain sections of the United States. With large population groups showing no such outbreaks over extended periods, the possibility of practically complete control has been clearly demonstrated. With sufficient attention to supervision of milk supplies, the numbers of milk-borne outbreaks in the United States should be materially reduced.

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Chapter 9

Pasteurization

In the marketing of milk and cream, or in their use in making various products, it often is desirable to destroy a portion or all of the contained organisms. Usually, this is accomplished by application of heat. Exposures high enough to effect complete destruction of the contained organisms are used with evaporated milk but are not employed with milk and cream for direct consumption or for most manufacturing purposes, because of the physical and chemical changes induced. However, lower exposures, with which there is a partial destruction of organisms, are extensively used and have been a significant factor in development of the dairy industry; when these lower exposures are employed under conditions which meet certain requirements, the process is referred to as pasteurization.

The objects of the pasteurization of dairy products are (1) to destroy any disease-producing organisms that may have gained entrance to them, and (2) to improve their keeping qualities. In the establishment of acceptable exposures to heat, two factors are involved, temperature and holding period, and one is as important as the other. A temperature which is ineffective if used for a short period may be entirely satisfactory when the period is prolonged. Cooling to 10 C (50 F), or preferably lower, immediately following the heating, to prevent growth of the organisms surviving, normally is regarded as a part of the process; however, when milk or cream is to be used for products involving growth of organisms, cooling need only be to the temperature employed for such development.

Pasteurization exposures required in various regulations differ somewhat but in all cases they assure destruction of the pathogenic organisms that sometimes are spread through milk. Usually, they are set on the basis of minimum requirements and these often are exceeded, particularly with milk and cream for manufacturing purposes and with ice-cream mix.

Origin. The word *pasteurization* is derived from the name of Louis Pasteur, a French scientist who developed the process. Pasteur wished to prevent abnormalities in wine, such as formation of acid, ropiness, etc., and found (1860 to 1864) that by heating the wine for a few minutes to a temperature of 50 to 60 C (122 to 140 F) the undesirable changes could be prevented. In 1868, heated wine was sent around the world successfully on the French frigate *La Sybile.* After the Franco-Prussian war Pasteur studied the spoilage of beer and found that heating also prevented undesirable changes in it. Pasteur was not the first to use heat to control fermentations. As early as 1782, Scheele heated vinegar to prevent spoilage. In 1810, Appert preserved various foods, including milk and cream, by application of heat to closed containers. Apparently, the heating of milk and cream for certain manufacturing purposes was practiced before the time of Pasteur. However, the studies of Pasteur were outstanding in that they were based on a knowledge of the role of organisms in causing the undesirable changes to be controlled.

In 1886, Soxhlet advised the heating of milk for infant feeding; the milk needed for a day was divided and that required for each feeding was put into a bottle of a type that could be easily cleaned and sterilized, after which the bottles were heated in hot water. Soxhlet incorrectly believed that milk treated according to this method was sterile. Heating of milk for general consumption and of cream for butter manufacture followed rapidly and pasteurization soon was well established in the dairy industry.

Types. With dairy products two main types of pasteurization are employed: (1) the low-temperature, long-time (LTLT) or holding method, and (2) the high-temperature, short-time (HTST) method which, before adequate control of the temperature and period of heating had been perfected, often was referred to as continuous or flash pasteurization.

With the LTLT method of pasteurization an exposure of at least 61.7 C (143 F) for at least 30 min often is required. The process commonly is carried out in vats or holding compartments. For a large operation a series of vats or holding compartments can be employed so that one vat or compartment always is supplying milk to the bottling equipment. They can be filled and emptied automatically. Pasteurization of milk and cream in bottles, which is referred to as final package pasteurization, is essentially a LTLT method. Its chief advantage lies in the elimination of contamination following the heating; however, with it, heating and cooling through the bottle wall is very slow and there may be variations in the temperature of the milk or cream in a bottle.

With the HTST method of pasteurization an exposure of at least 71.1 C (160 F) for at least 15 sec commonly is required. The short holding period permits the development of equipment to which, and from which, the milk flows in a continuous stream. At present plate, tube, and electric heaters are in use. For certain products, especially cream for butter, the exposures used are relatively high.

At one time regulatory officials did not accept HTST pasteurization for market milk because of irregularities in the heating and the tendency to use inadequate exposures in an attempt to avoid overheating portions of the milk. Now, it is generally accepted because the equipment has been so improved that heating of the milk is very uniform and underheating of a portion of it is avoided by flow diversion valves.

Regenerative systems, by which the incoming milk is heated and the pasteurized milk cooled through an exchange of heat between them, are useful when large volumes of milk are being treated with either process. HTST pasteurization rather regularly makes use of the regenerative system.

Basis for present pasteurization exposures. Three effects of the heat applied to milk must be taken into account whenever a pasteurization exposure is under consideration: (1) bacterial efficiency, (2) influence on flavor, and (3) influence on cream line. If bacterial efficiency were the only consideration, relatively high exposures might be advisable but the effect of heat on flavor and cream line requires that the exposures be reasonably low. In general, exposures are selected for market milk that ensure destruction of any pathogens present and have as little influence as possible on the physical and chemical properties of milk. A slight heated flavor in market milk is not objectionable to many people and is preferred by some; also, it tends to mask certain off flavors, such as slight feed and absorbed flavors. However, with good raw-milk supplies, the general tendency is to give the milk just as little heated flavor as possible through adequate control of the heat treatment.

With various products other than milk it is less essential to consider the influence of heat on the physical and chemical properties and, in some instances, conspicuous effects along these lines may be desirable.

Bacterial efficiency. To be satisfactory from the standpoint of bacterial efficiency, pasteurization must kill a reasonable percentage of the total bacteria present, including all pathogens. Bacterial efficiency can be expressed as the percentage of the total bacteria that are killed during the heating, but this is not entirely satisfactory because, with excessive numbers of bacteria present originally, a high percentage may be destroyed by exposures that still leave many organisms and do not kill all pathogens. In general, with a given exposure, the higher the initial count the greater will be the percentage of organisms destroyed because high counts on raw milk usually include many *S. lactis* organisms that are easily killed by heat; however, there are numerous exceptions to this relationship. Requirements dealing with destruction of bacteria during pasteurization ordinarily refer to the numbers permitted both before and after the heating.

Destruction of pathogens that may be in milk is the important point to consider with reference to the bacterial efficiency of a pasteurization exposure. Because *M. tuberculosis* is more resistant to heat than other pathogens likely to be present in milk, its resistance usually is used as a basis in establishing satisfactory exposures.

Heat resistance of *M. tuberculosis*. The early investigations on the exposure to heat required to destroy *M. tuberculosis* in milk yielded variable results,[1] with the findings of one investigator often showing pronounced variations. In 1899, Smith[2] explained this by pointing out that formation of a scum or pellicle on the milk, due to evaporation, enabled the organism to resist exposures that otherwise would destroy it. He found that, when suspended in milk, *M. tuberculosis* was killed at 60 C (140 F) in 15 min if no pellicle was allowed to form, but with a pellicle it might survive 60 C for 60 min. Russell and Hastings[3] reported that heating tuberculous milk in a tightly closed commercial pasteurizer at 60 C for 10 min destroyed *M. tuberculosis;* they recommended an exposure of 60 C for not less than 20 min in order to ensure thorough pasteurization. Later, these investigators[4] reported results obtained with short periods of heating and concluded that 71.1 C (160 F), or above, for 1 min suffices to destroy the bovine cultures so that the disease is not produced in guinea pigs inoculated with organisms in amounts ranging from 2 to 5 mg. Rosenau[5] found that the organism in milk was killed when heated at 60 C for 20 min or at 65 C (149 F) for a much shorter period.

Studies of Traum and Hart[6] are particularly significant since they involved milk from a heavily infected herd that was intended for consumption after commercial pasteurization. The reacting animals, which at one time numbered 500, were kept separate, and the milk from them was sold after it had been pasteurized. Pasteurization was carried out in three well-insulated vats. Steam was turned into the circulating water as soon as milk began to flow into a vat and 20 to 30 min were required to fill the vat and bring the milk to the pasteurizing temperature; emptying the vat required 15 or 20 min, so that the last milk leaving the vat received considerable additional exposure before running over the cooler. The temperatures were obtained with a recording thermometer, but part of the time a thermometer also was placed directly in the milk. The milk was examined at a distant point, within 24 to 48 hr of collection, by centrifuging 90 to 95 ml and inoculating the cream and sediment subcutaneously or intramuscularly into two or three guinea pigs; reinoculations were made in all cases of suspicious lesions, even when tubercle bacilli were not demonstrated microscopically. Of the 24 samples of raw milk studied, all but 1 produced tuberculosis in inoculated animals and with it the guinea pigs died of septicemia; with 2 samples 1 animal developed tuberculosis while the other remained well; with 3 samples 1 animal developed tuberculosis while the other died soon after inoculation. After pasteurization, 23 samples failed to produce tuberculosis or cause death in a single instance; 12 of the samples were heated above 60 C for longer than 20 min, whereas the remaining 11 were heated to 60 C and held for only 20 min.

Bartlett[7] reported that pasteurization at 61.1 to 64.4 C (142 to 148 F) for 30 min under commercial conditions destroyed *M. tuberculosis* in milk. Campbell-Brown[8] studied 25 strains of *M. tuberculosis* and found no wide differences in thermal death points; human and bovine organisms appeared to be similar in resistance. The investigator concluded that at 60 C it requires 20 min to prevent infected milk from causing the disease in guinea pigs; at 70 C (158 F) it requires 5 min. In the studies carried out by Cameron-Macaulay,[9] *M. tuberculosis* from sputum and from tuberculous glands was destroyed by heating in milk at 62.8 to 65.6 C (145 to 150 F) for 30 min. Jenkins[10] found that the organism in naturally infected milk was killed at 60 to 62.8 C for 30 min.

In studies carried out since Smith recognized the importance of preventing pellicle formation on the milk being heated, exposures generally considered to destroy *M. tuberculosis* have occasionally failed to do so. White[11] injected 30 guinea pigs with naturally infected milk after heating it to 62.5 C (144.5 F) for 30 min and noted 1 positive result; he stated that it was possible an accident had occurred. Meanwell[12] carried out a series of tests with naturally infected milk and obtained data as follows: When injected with milk heated at 62.8 C for 30 min, 136 pigs were negative and 2 positive; with milk heated to 60 C for 30 min, 96 pigs were negative and 0 positive; with milk heated to 60 C for 20 min, 50 pigs were negative and 2 positive; with milk heated to 59.3 C (138.8 F) for 20 min, 3 pigs were negative and 10 positive.

In extensive trials carried out by a group of investigators[13] working with commercial equipment, the bovine organism was destroyed in some of the tests in three types of improved commercial pasteurizers at 58.9 C (138 F) for 30 min. The studies indicated the necessity of designing pasteurizers properly if destruction of pathogens is to be accomplished under practical conditions.

Park[14] reported that the following exposures were required to kill *M. tuberculosis* in milk:

55.6 C (132 F)	60 min
56.7 C (134 F)	40 min
57.8 C (136 F)	30 min
58.9 C (138 F)	20 min
60 C (140 F)	15 min
61.1 C (142 F)	10 min
62.8 C (145 F)	6 min
65.6 C (150 F)	2 min
68.3 C (155 F)	1 min
71.1 C (160 F)	30 sec
76.7 C (170 F)	20 sec

He also noted that of 100 samples of ordinary milk 20 per cent contained the organism before pasteurization and none after pasteurization. Oldenbusch *et al.*[15] found that *M. tuberculosis* (bovine) was killed in cream in less than 20 min at 57.2 C (135 F), in less than 10 min at 60 C, in less than 7 min at 61.1 to 61.9 C (142 to 143.5 F), and in less than 3 min at 62.8 C. In ice-cream mix the organism was killed within 6 min at 62.8 C and within 3 min at 65.6 C. Minett and Pullinger[16] noted that every one of 43 samples of milk coming into London in 3,000-gal tanks contained *M. tuberculosis,* while the same number of samples after pasteurization were all negative. Price[17] found in Canada that 26 per cent of 100 samples of mixed raw milk contained *M. tuberculosis* and all of 100 samples examined immediately after pasteurization were negative.

Mattick and Hiscox[18] reported the following test: When a commercial milk plant was temporarily out of use, the cast-plate heat exchanger normally employed to heat milk to 62.8 C for holding pasteurization was used as a plate heat exchanger for HTST pasteurization. Using the control apparatus already installed, temperatures of about 72.2 C (162 F) were aimed at but there was no precision control equipment. A length of pipe leading from the heating to the regenerating sections of the exchanger provided a holding period of 11.5 sec. The total period in the heating section was about 16 sec. At intervals, raw and pasteurized milk were taken at such times that they represented the same incoming milk. Of 37 samples of raw milk, 28 contained *M. tuber-*

culosis on guinea pig inoculation. No pasteurized milk was positive, even when the temperature of heating was only 70.3 C (158.5 F) and the temperature of holding was 70 C (158 F). The temperature drop in the "holding" pipe was consistently about 0.28 C (0.50 F). The results show that *M. tuberculosis,* in the numbers in which it occurs in naturally infected milk, is killed in milk held at 70 C for 11.5 sec after being in the regenerating and heating sections, at a temperature culminating at 70.3 C, about 16 sec.

Heat resistance of *Brucella* organisms. Rosenau[5] noted that *Br. melitensis* was not destroyed at 55 C (131 F) for a short time, but was killed at 60 C (140 F) and the majority of the organisms were dead at 58 C (136.4 F). Evans[19] reported that 51.7 C (125 F) for 30 min or 62.8 C (145 F) for 30 sec killed *Br. abortus.* Carpenter and Boak[20] noted that human, porcine, and bovine cultures of *Brucella* were not viable after an exposure to 60 C for 20 min. Guinea pig inoculations were used in testing the heated organisms and were found to be more reliable than cultures; in a few instances cultures were negative when inoculated guinea pigs became infected. Park[21] found that when heated in milk *Br. melitensis* and *Br. abortus* survived 60 C for 7.5 min but were killed in 10 min; at 61.1 C (142 F) they survived 5 min but were killed in 7.5 min; at 62.8 C they were killed in 5 min. Carpenter and Boak[22] reported that 20.4 per cent of 122 samples of raw milk from 67 villages and 2 cities showed evidence of *Br. abortus* infection when 2 ml of cream from each sample was injected subcutaneously into guinea pigs, whereas all of 205 samples of pasteurized milk and cream from 12 villages and 38 cities were negative. Temperatures of 61.1 and 62.8 C for 20 or 30 min were satisfactory for pasteurizing milk containing the most virulent strains of *Br. abortus.* Murray *et al.,*[23] using a vat pasteurizer, found that a temperature of 62–63 C (143.6–145.4 F) for 3 min was sufficient to destroy *Brucella* organisms, both the bovine and porcine varieties. With the cover of the vat open, a much longer time was required and the results were irregular; viable organisms were recovered from the foam even after 30 min heating. The necessity of a flush valve was indicated since with an ordinary faucet outlet viable organisms were obtained after 30 min heating. Park *et al.*[24] reported that two strains of *Br. suis* in hermetically sealed tubes of whole milk (500,000,000 organisms per milliliter) were non-viable after 20 min at 60 C, after 15 min at 61.1 C, or after 7 min at 62.2 C (144 F). The same strains were more resistant in cotton-stoppered tubes of milk and survived 30 min at 62.2 C in milk containing 10,000,000 to 500,000,000 per milliliter but did not survive when the milk contained 5,000 to

1,000,000. In London,[16] 27 of 43 samples of raw milk from 3,000-gal tanks contained *Br. abortus* and the same number of samples of pasteurized milk were negative.

Heat resistance of additional pathogens. Rosenau[5] found that *E. typhosa* was killed in milk heated to 60 C (140 F) and maintained at this temperature for 2 min; the great majority of the organisms was killed by the time the milk reached 59 C (138.2 F), and only a few survived at 60 C. *C. diphtheriae* often failed to grow after the milk reached 55 C (131 F), although occasionally it survived until the milk reached 60 C. The resistance of *V. comma* was similar to that of *C. diphtheriae;* it usually was destroyed when the milk reached 55 C but once it lived until 60 C was reached. The dysentery organisms (Shiga and Flexner) withstood 60 C for 5 min, but were killed at this temperature for 10 min. Rosenow and Hess[25] found that an organism responsible for an outbreak of septic sore throat was destroyed at 60 C for 20 min.

Krumwiede and Noble[26] investigated the heat resistance in milk of 27 *E. typhosa* cultures recently isolated from carriers, 7 paratyphoid A cultures, 12 paratyphoid B cultures, and 4 *Salmonella enteritidis* cultures. The milk was sterilized, cooled, and tested for sterility. It then was heated to 60 C in a water bath, suspensions of the organisms in salt solution added, and rubber stoppers inserted. After being shaken it was returned to the water bath and held at 60 C for 15 min. After rapid cooling, the milk was held 48 hr at 37 C (98.6 F) to allow multiplication of any organisms that survived. None of the cultures yielded growth.

In trials carried out with commercial pasteurizers,[13] *E. typhosa* was destroyed in one series of tests by momentary heating in a commercial heater to 60.6 C (141 F) and by one type of commercial holder at 55.6 C (132 F) for 30 min; *C. diphtheriae* and hemolytic streptococci were destroyed by momentary heating in a commercial heater to 53.9 C (129 F). Park[21] reported that 200 strains of hemolytic streptococci obtained from different cases of septic sore throat, scarlet fever, erysipelas, and other diseases known to be due to hemolytic streptococci were all killed at 60 C for 30 min when heated in milk. The majority of them was killed at 57.8 C (136 F) for 30 min. Oldenbusch *et al.*[15] found that two strains of streptococci, one from scarlet fever and one from septic sore throat, did not survive in cream at 57.2 C (135 F) for 5 min or at 60 to 61.1 C (140 to 142 F) for 1 min. *E. typhosa* was killed at 57.2 C in less than 10 min, at 60 C in less than 5 min, and at 61.1 to 62.8 C in less than 3 min. In ice-cream mix the streptococci and *E. typhosa* were killed in less than 5 min at 62.8 to 65.6 C (145 to 150 F) and *C. diphtheriae* did not survive these temperatures for 0.5 min.

Margin of safety in pasteurization. Under plant conditions temperature and time often cannot be controlled so accurately as in the laboratory; temperature especially may deviate from what is intended as a result of variations in different parts of the pasteurizer, inaccuracies in thermometers, and failure to adjust the heating carefully. This necessitates inclusion of a considerable margin of safety in the pasteurization exposure if complete destruction of pathogens that may be present in milk or cream is to be assured. As a result, exposures set by various regulations governing commercial pasteurization always are above those required for the destruction of *M. tuberculosis* in well-controlled tests.

Influence of pellicle formation on heat resistance of organisms. The protection given *M. tuberculosis* by the pellicle formed on heated milk undoubtedly also occurs with other organisms. Russell and Hastings[27] inoculated milk with a coccus and found that the pellicle that developed on heating the milk yielded growth when the milk below did not. Removal of a pellicle resulted in formation of another, but this was sterile. When a pellicle containing organisms was heated in water, the organisms resisted temperatures that ordinarily killed them. No appreciable diminution in temperature could be detected at the surface of heated milk on which a pellicle was forming.

Increased resistance of organisms in the pellicle developed on heated milk evidently is due to more than one factor. The pellicle forms as a result of evaporation and this requires heat so that the temperature undoubtedly is lowered, even if no decrease can be detected by ordinary measurements. It is probable also that the pellicle offers some protection, either by its resistance to penetration of heat or in some other way. The influence of pellicle formation on survival of organisms during pasteurization is of importance under practical conditions and pellicle development should be prevented, either by agitating the material or by heating and holding under conditions which prevent evaporation.

Importance of uniform heating. Reasonable uniformity in both temperature and period of heating of all portions of a lot of milk or cream is highly desirable. It is essential that all the material be heated sufficiently to destroy any pathogens present, whereas overheating of portions may give a pronounced heated flavor and, in the case of milk, a serious decrease in the cream line.

Uniform heating of a large volume of milk or cream presents a difficult engineering problem and various types of pasteurization equipment have defects of one sort or another. Some of the common causes of underheating a portion of a lot of milk or cream are (1) leaky inlet or outlet valves which permit some of the material to escape the desired heat exposure, (2) dead ends which retain material in such a way that it does not mix properly with that coming in contact with the heating surface, (3) splashing that carries material to parts of the equipment where it does not receive sufficient heating, and (4) formation of foam through which heat is transferred only slowly. Overheating may result from (1) high temperature heating surface which supplies heat so rapidly that the material next to it is heated above the temperature desired for the entire lot, and (2) unnecessarily long holding, such as occurs when the equipment heats or cools so slowly that the material is near the pasteurization temperature for extended periods.

Formation of foam on milk during pasteurization is of considerable bacteriological significance. Whittaker *et al.*[28] studied the temperatures of milk and foam during commercial pasteurization by means of thermocouples and found, in 19 runs, that the temperature of the foam always was lower than that of the corresponding milk. The average temperature of the foam was 2.8 C (5 F), or more, below that of the milk in 84.2 per cent of the tests at the beginning of the holding period, in 73.3 per cent of the tests at the middle of the holding period, and in 84.6 per cent of the tests at the end of the holding period. Temperature of the foam frequently was more than 6.1 C (11 F) below that of the milk and in one instance was 10.1 C (18.1 F) below. The foam and corresponding milk were examined for numbers of bacteria, and at both beginning and end of the holding the foam showed the higher count in every instance. Although the milk gave the expected decrease in number of bacteria during holding, 66.7 per cent of the foam samples gave a higher count after holding than before; the average increase in these samples was 176.3 per cent. There was reduction in the bacterial content of the foam in vats when the covers were tightly closed, but in vats with the covers open or loose fitting there was an increase; in agreement with this, the average temperature of the foam increased approximately 1.7 C (3 F) during the holding in vats with the covers tightly closed, whereas there was no increase when the covers were raised. At both beginning and end of the holding there were wide variations in bacterial contents of samples of foam from different parts of a vat. The butter fat content of foam varied from that of the corresponding milk and was higher in nearly all cases; at the end of the holding period the percentage of fat in the foam usually was higher than at the beginning.

The danger of underheating a portion of the milk or cream during pasteurization is now recognized in the design of dairy equipment, and flush valves, non-leak valves, foam heaters, and other refinements have been introduced.

Cooling after pasteurization. The necessity of cooling milk and cream to a low temperature immediately after pasteurization is generally appreciated and is demanded by many regulations. Pasteurization destroys a large percentage of the bacteria present but cannot prevent growth of those remaining, or of those that gain entrance during the subsequent handling. Activity of such organisms must be prevented by low temperatures and, from the standpoint of control of bacterial growth, these should be reached quickly.

Studies of Ayers and Johnson [29] indicate that the sudden chilling of organisms surviving pasteurization has no effect on them. Milk was pasteurized in sterile flasks at either 62.8 or 71.1 C (145 or 160 F) for 30 min and then cooled rapidly by running it through a sterile copper coil surrounded by brine; in each trial bacterial counts before and after cooling were essentially the same.

Bacteria surviving pasteurization. Pasteurization normally does not destroy all the organisms in a lot of milk and the heat-resistant, or thermoduric, types remain (Hileman).[30] These belong to various physiologic and morphologic groups. When pasteurization was being rapidly extended in the field of dairying, the prevailing idea was that bacteria that survived the process were largely spore-forming species. This view came, in part, from studies on the organisms present in milk heated to temperatures much higher than those employed in pasteurization. Investigations have shown that certain non-spore-forming bacteria are capable of resisting exposures accepted as satisfactory; they constitute a much larger percentage of the flora of pasteurized milk than do the spore formers and play an important role in the bacterial changes in pasteurized milk. Ayers and Johnson [29] found that of 225 unselected cultures which survived pasteurization at 62.8 C (145 F) for 30 min, only 3 (1.35%) formed spores. Macy [31] reported that, among 81 cultures isolated from laboratory pasteurized milk, there were 13 spore-forming rods, 19 streptococci, 24 micrococci, 2 sarcinae, and 23 non-spore-forming rods.

Streptococcus organisms. Various streptococci are capable of surviving pasteurization. Ayers and Johnson [32] studied 139 cultures of streptococci isolated from cow feces, the udder, and the mouth of the cow, and from milk and cream, and found that, with a 30-min exposure in milk, 89 or 64.03 per cent survived 60 C (140 F), 46 or 33.07 per cent survived 62.8 C (145 F), and 3 or 2.56 per cent survived 71.1 C (160 F); all were destroyed at 73.9 C (165 F). The 22 cultures that formed long chains were much less resistant to heat than the 117 cultures that did not. Sherman and Stark [33] investigated 294 cultures of streptococci which grew actively at 45 C (113 F) and noted that each of the species included survived 30 min at 62.8 C in milk. *S. thermophilus* commonly is present in pasteurized milk. This species is rarely encountered in the isolation of lactic acid organisms from raw milk, either sweet or sour, owing probably to its slow growth in competition with *S. lactis* at the common holding temperatures. *S. liquefaciens* is relatively resistant to heat and undoubtedly often survives pasteurization. Hammer and Baker [34] found that with a heating period of 30 min a considerable number of the *S. paracitrovorus* cultures studied survived 60 C, whereas a somewhat smaller number survived 65 C (149 F); one culture survived 75 C (167 F).

Micrococcus organisms. Hammer and Trout [35] noted that cocci forming yellow colonies often were present on plates poured with dairy products, especially those that had been subjected to heat, and that these organisms ordinarily resisted the usual pasteurization exposures for market milk; they were resistant in skim milk, whole milk, cream, bouillon, and ice-cream mix, but the resistance in milk was decreased in the presence of sufficient acid to cause coagulation. The yellow cocci studied belonged to several types. Hucker [36] found micrococci often survived pasteurization, the most frequently occurring species being *M. epidermidis, M. candidus, M. varians,* and *M. luteus.* Various other investigations indicate the importance of this morphologic group in pasteurized milk.

Escherichia-Aerobacter organisms. Although various studies indicate the *Escherichia-Aerobacter* organisms are not very resistant to heat, this type sometimes survives pasteurization. Ayers and Johnson [37] studied the heat rèsistance of 174 cultures isolated from cow feces, human feces, flies, milk, cream, and cheese, using a heating period of 30 min, and found that 95 or 54.5 per cent survived 60 C (140 F), whereas 12 or 6.89 per cent survived 62.8 C (145 F). More recent studies also emphasize the possibility of certain *Escherichia-Aerobacter* organisms surviving pasteurization. Tanner and Windsor [38] found 1 of 23 cultures of *E. coli* repeatedly survived heating in sealed tubes at 62.8 C for 30 min. In the examination of 100 samples of milk, Beavens [39] reported that with 32 of them *Escherichia-Aerobacter* organisms survived the temperatures used in commercial pasteurization. Stark and Patterson[40] studied 505 cultures of *Escherichia-Aerobacter* organisms recently isolated from water, raw and pasteurized milk, and human and bovine feces. Of these, 487 (96%) failed to survive 60 C for 30 min; 14 of the remaining 18 were destroyed by 61.7 C (143 F) for 30 min; and the other 4 were destroyed by 62.8 C for 30 min.

Long *et al.*[41] found that *Escherichia* cultures of comparatively high heat resistance were rather frequently encountered in dairy products, especially butter. Of 116 cultures selected from isolations from butter, 74 (63.8%) survived 20 min at 61.7 C, 48 (41.4%) survived 30 min, and 28 (24.1%) survived 40 min. Counts on certain of the resistant cultures indicated that in some of them appreciable numbers of cells still were present after 50 min at 61.7 C. Heat-resistant *Aerobacter* cultures were not encountered. The authors suggested that the heat treatment given churns in cleaning and sterilizing may tend to select the more resistant types of coliform organisms which then multiply and are added to butter during the churning process. Craige[42] reported that 8 strains each of *Escherichia,* intermediate, and *Aerobacter* coliform organisms isolated from raw milk, pasteurized milk, and ice cream all survived 62 C (143.6 F) for 30 min in milk when present in sufficient number. No strain tested survived when the concentration before pasteurization was less than 700 per milliliter. Craige considered that one of the causes of coliform organisms in pasteurized milk is excessive numbers in the raw milk.

According to Tiedeman and Smith,[43] much of the pasteurized milk on the market is not completely free of coliform organisms, particularly during summer. The evidence indicated that freedom from coliform organisms in 1-ml portions of pasteurized milk is an attainable goal in well-operated plants.

Lehmkuhl[44] concluded that milk cans, whether acid- or alkali-washed, may carry coliform contamination through the washing steps, particularly if milk residues are not completely removed. Efficient mechanical operation, use of sound cans, and sufficiently high sterilizing temperatures determine whether coliform organisms will survive the washing process.

Heat resistance of coliform organisms is of special importance because coliform organisms in a pasteurized dairy product sometimes are considered to result from inefficient pasteurization or recontamination. Frequently, this is the case but there is ample evidence that certain coliform cultures can resist the usual pasteurization exposures and are present in pasteurized milk, etc., because they survived the heat treatment. This suggests that coliform organisms found in a pasteurized product should be tested for heat resistance before drawing definite conclusions as to their significance. In such tests there definitely is a greater chance for the survival of an occasional bacterial cell with large numbers in the material being heated than with small numbers, and for detailed information plate counts before and after pasteurization are advisable. Large numbers of coliform organisms in pasteurized milk, etc., may be due to growth at relatively low temperatures, as well as to extensive contamination or survival.

Lactobacillus organisms. Because lactobacilli appear to be of importance in the ripening of cheddar cheese, their heat resistance is of significance in connection with the manufacture of cheese from pasteurized milk. In the work of Evans *et al.*,[45] *L. casei* regularly was present in cheese made from pasteurized milk, which suggests that it resists pasteurization; it usually developed more slowly in pasteurized-milk cheese than in raw-milk cheese so that the heating may have killed some of the organisms.

General considerations. From the data available, it is evident that a variety of organisms sometimes survive the usual pasteurization exposures used with milk. An important point in this connection is the failure of *S. lactis* to survive; as a result this species cannot greatly influence the changes that occur when pasteurized milk undergoes fermentation and various organisms may be active.

Low bacterial efficiencies. In some instances a pasteurization exposure that is expected to give a satisfactory efficiency fails to do so because the milk contains relatively large numbers of thermoduric organisms.

Dotterrer [46] occasionally found high bacterial counts on milk from certain pasteurizing plants. In studies on milk from individual farms he noted great variations in the percentages of organisms destroyed by pasteurization and in the numbers per milliliter of the pasteurized milk. Heat-resistant organisms were traced to improperly handled utensils, including milking machines.

Hussong and Hammer [47] studied the heat resistance of the organisms in milk from various farms by pasteurizing small amounts in sealed tubes with an exposure of 61.1 C (142 F) for 30 min. Wide variations were noted in the efficiencies obtained and there also were variations with the samples from a given farm. Milk from one farm showed low efficiencies and high counts on the pasteurized milk over an extended period. Better methods of producing and handling the milk resulted in higher efficiencies and lower initial counts, so that counts on pasteurized milk were markedly decreased; when the initial counts then increased, owing to hot weather, the higher efficiencies and lower counts on the pasteurized milk persisted. Considering the samples from the various farms, there was no close relationship between efficiencies and initial counts, and both high and low efficiencies were obtained on milk with high and with low initial counts; however, with the samples from some farms a tendency for high efficiencies to accompany high initial counts and low efficiencies to accompany low initial counts was evident.

Anderson and Meanwell,[48] using 62.8 to 63.3 C (145 to 146 F) for 30 min, also noted great variations in numbers of heat-resistant organisms in milk from different sources. The resistant organisms frequently originated from unsterile utensils, and non-cooling of milk at the farms often encouraged their development. Under the conditions studied, daily sterilization of milk utensils largely eliminated heat-resistant organisms from the milk.

Macy[31] reported that counts made on milk delivered by 274 patrons to six milk plants revealed that 33.6 per cent of the patrons more or less regularly delivered raw milk that was difficult to pasteurize satisfactorily. Some of the plants with the larger numbers of such patrons had been having difficulty in meeting the requirements for pasteurized milk.

Factors influencing heat resistance of organisms. Resistance of a bacterial species to heat is not a sharply defined character that can be determined exactly. Variations in resistance occur with different cultures belonging to a species, and also with the individual organisms in a culture. Ordinarily, some cells in a culture retain their vitality at exposures definitely higher than those required for the destruction of others, and these relatively resistant cells may permit a culture to survive comparatively high exposures. In addition to the normal variation in heat resistance, certain factors have a definite influence on it.

pH. Mattick and Nichols[49] studied the effect of the *p*H of milk on destruction of organisms by heat. Each sample was well mixed and divided into five portions. One portion was used as a control and small amounts of sodium hydroxide or hydrochloric acid were added to the others. After the samples had come to equilibrium, the *p*H values were determined and the samples pasteurized at 62.8 C (145 F) for 30 min. Each sample was plated immediately before and after pasteurization. Variations in the *p*H values resulted in differences in destruction of organisms by the heat. As the *p*H values decreased the numbers of bacteria surviving also decreased. The effects were much greater with some samples of milk than with others, which may have been due to differences in the floras.

Fat content. In tests on certain non-spore-forming bacteria of relatively low heat resistance, Nichols[50] found that an increase in the butterfat content of the medium failed to increase the heat resistance.

Age of bacterial cells. Sherman and Albus[51] noted that young cells are more easily killed by various agents, including heat, than are old cells. Robertson[52] reported that young, rapidly growing, or adolescent cells of three bacterial species were more susceptible to destruction by heat than older cells. Using pure cultures of three organisms important in dairy products, Stark and Stark[53] found that young cells were more rapidly destroyed at pasteurization exposures than were old cells. Sherman *et al.*[54] reported that milk held at temperatures permitting bacterial growth, such as 15.5 C (59.9 F), showed a greater percentage destruction of bacteria on pasteurization than similar milk when fresh or when held at such temperatures as 4.4 C (40 F) that are too low for active multiplication of the bacteria involved. Fabian and Coulter[55] found that the susceptibility of bacterial cells to heat is greatly influenced by their age, young cells being more rapidly killed than older ones. Hammer and Hussong[56] noted that young cells of certain organisms producing ropiness may be more easily destroyed at pasteurization temperatures than old cells.

With *E. coli,* Elliker and Frazier[57] found that during the period of most active reproduction at both 28 and 38.5 C (82.4 and 101.3 F) the heat resistance declined to its lowest point. As the cultures entered the maximum stationary growth phase, their heat resistance again rose. At the higher temperatures the maximum percentage survival during the maximum stationary growth phase exceeded that at temperatures below the optimum.

In connection with the age of bacterial cells, the growth temperature is important. Commonly, organisms are more heat resistant when grown at their optimum than when grown at lower temperatures. With certain organisms, however, growth at temperatures below the optimum apparently results in increased resistance. A *Streptococcus* which showed increased resistance to heat as its temperature of growth was reduced below the optimum was described by Anderson and Meanwell;[58] two cultures of streptococci increased in heat resistance as a result of 24 hr storage at 3 to 4 C (37.4 to 39.2 F). Claydon[59] found that at 10 C (50 F) *S. lactis* cultures grew more slowly than at higher temperatures but attained higher thermal resistance; the highest resistance noted with a culture grown at 10 C was 13 min at 61.7 C (143 F).

Data indicating that young bacterial cells are more easily killed than older cells have led to attempts to obtain lower bacterial counts on pasteurized milk by holding the raw milk for a short period at a temperature relatively favorable for the growth of bacteria before heat treating. In general, these have not been successful; presumably, such factors as variations in optimum growth temperatures of the species present, differences in the effects on various organisms of growth below the optimum, etc., result in relationships other than those expected on the basis of tests with pure cultures.

Special considerations. There is some evidence that an exposure to heat which is slightly below that required to kill an organism may so modify it that one or more characters, especially physiological characters, are different from what they were originally. When results suggesting such a change are obtained, they often are not accepted and the irregularity is attributed to contamination. However, variations in organisms have been noted as a result of various treatments, so that a character modification as a result of an almost fatal exposure to heat might be expected.

In the course of pasteurization experiments with pure strains of coliform organisms inoculated into sterile milk, Katzin *et al.*[60] made post-pasteurization counts by means of the most probable number technic, using tubes of lactose broth. Heavy growth sometimes was noted without gas formation. A number of such growths investigated proved to be Gram-negative rods. A few reverted to lactose fermentation under cultivation, confirming their identity as coliform organisms. Other strains retained various degrees of impairment of lactose and dextrose fermentation during the short period of observation but otherwise were typical.

Detection of thermoduric organisms. Since relatively large numbers of thermoduric organisms in raw milk may result in excessive plate counts on the pasteurized product, milk from individual herds often is tested for heat resistance of the flora. Samples can be laboratory pasteurized in tubes with the LTLT method and plate counts made before and after, or only after, heat treatment. Counts also can be made with the Burri technic or the oval tube technic. Streaking a 0.001-ml loop of the heated milk on agar in a plate, using several samples to a plate, also is satisfactory. Pasteurization of many small portions of milk with the HTST method presents difficulties, unless relatively large errors in the exposure are accepted.

A microscopic procedure for detecting thermoduric organisms in milk was suggested by Mallman *et al.*[61] With it, 5 to 10 ml of milk is held at 58 to 60 C (136.4 to 140 F) for 2 hr and a microscopic count made. Samples showing 40,000 or more bacteria per milliliter are considered to contain excessive numbers of thermoduric organisms. The authors noted that microscopic counts on pasteurized milk directly after pasteurization represent living and dead bacteria; the incubation at 58 to 60 C destroys non-thermoduric organisms and causes their dissolution so that a microscopic count after the incubation represents viable thermoduric bacteria.

Bryan and Bortree [62] determined the presence of heat-resistant bacteria in milk by pasteurizing the milk-agar mixture after making a Frost little plate and then making a second little plate for the pasteurized milk count. Results compared favorably with those of the pasteurization test in a series of 1,375 milk samples; they were ready within 5 hr.

In a comparison of various methods of detecting thermoduric organisms in milk, Fischer and Johns [63] found that the oval tube technic gave results in much closer agreement with the plate count than did the microscopic procedures tested. Macy and Erekson [64] reported a close correlation between results of the Burri technic and the standard plate count.

Parfitt [65] noted that, in general, there is no relation between methylene blue reduction and the thermoduric population of milk. He considered that one of the reasons for high thermoduric counts is that shippers have found short cuts in methods which are not detected by the methylene blue test.

Sources of thermoduric organisms. Studies on sources of thermoduric organisms in milk have emphasized the importance of contamination on the farms, particularly from utensils and equipment that have not been properly cleaned. Many instances of high thermoduric counts on raw milk have been corrected by improving the condition of farm utensils and equipment, especially milking machines. Inadequate care of utensils and equipment may permit a build-up of a relatively heat-resistant flora in the corners and crevices, particularly when temperature and moisture conditions favor active development of organisms.

Dotterrer [66] noted that the immediate source of thermoduric organisms in milk generally is farm utensils, with milking machines involved more often than other equipment, and that a majority of thermoduric bacteria in milk are micrococci, many of which probably come originally from the cow's udder.

Parfitt[65] reported the following results, showing that, with the 104 milk shippers involved, machine-drawn milk had relatively high counts of thermoduric organisms:

COMPARISON OF GENERAL NUMBERS OF THERMODURIC ORGANISMS IN MILK WITH HAND AND WITH MACHINE MILKING

Thermoduric Organisms per Milliliter (In Thousands)	Number of Shippers Using	
	Hand Milking	*Machine Milking*
below 1	26	0
1 to 5	20	0
5 to 10	7	0
10 to 20	1	23
20 to 30	0	5
30 to 40	0	2
40 to 50	0	12
over 50	0	8
Total	54	50

The build-up of thermoduric organisms in milking machines and the need for adequate care of the machines are evident from the following data reported by Parfitt:[65]

BUILD-UP OF THERMODURIC ORGANISMS IN MILKING MACHINES

Days after Sterilization	Total Bacteria per Milliliter of Milk	Per Cent Thermoduric Bacteria
0	325	0.1
1	2,200	1.0
2	11,150	6.1
4	16,350	13.0
5	6,000	9.0
6	19,900	30.0
8	12,200	40.0
9	22,000	40.0
11	19,000	50.0
12	38,200	57.0

Bryan *et al.*[67] reported that properly pasteurized milk (phosphatase-negative) from a small dairy increased in count during a 4-mo period from its normal level of 15,000 per milliliter to as high as 2,500,000. Despite proper plant sanitation, the high counts persisted. The immediate source of the high counts of heat-resistant bacteria was the milk of one of the seven producers. An unclean milking machine contributed heat-resistant organisms directly to the milk during the milking and also served to inoculate the cows' udders with the organisms. After the equipment was properly cleaned and sanitized, the cows rid themselves of the heat-resistant bacteria in from 1 to 4 mo. During this same period the standard plate count of the properly pasteurized milk decreased to its previous normal level of 10,000 to 25,000 per milliliter.

Although pails, filters, coolers, and other equipment on farms are of less importance than milking machines as a source of thermoduric organisms, they sometimes are responsible for excessive numbers and should be considered whenever high thermoduric counts are obtained. Their significance is indicated by the fact that milk high in thermoduric organisms may come from farms on which milking is done by hand.

In the treatment of utensils responsible for high thermoduric counts the results of Maack and Prucha[68] are of significance. They found that heat-resistant organisms also were resistant to chlorine. One non-spore-forming species that required 61.7 C (143 F) for 3.5 hr to kill all the bacterial cells required 100 ppm chlorine for 2 min or 20 ppm for 5 min for its destruction.

Seasonal variation. Macy and Erekson[64] studied the seasonal variation in thermoduric organisms, using laboratory pasteurization on 7,562 samples, and found the results for the spring and the autumn were quite similar but there were differences between the summer and the winter. In the summer there were many samples, even in the higher qualities of milk, that did not pasteurize satisfactorily; this was in marked contrast with the winter. Percentages of samples showing counts below 5,000 per milliliter after pasteurization were 80.1 per cent in winter and 40.5 per cent in summer. The data indicate there is a much better chance that high-quality milk can be pasteurized satisfactorily than poor-quality milk but an appreciable number of samples of low-count raw milk can be reduced to below 5,000 bacteria per milliliter only with difficulty.

Contamination in distributing plants. Thermoduric organisms may be added to milk in the distributing plant as a result of their accumulation in plant equipment through faulty cleaning and sterilization. Milkstone often appears to be a factor in such contamination. Repasteurization of milk may be responsible for a build-up of thermoduric organisms. The distributing plant also has a responsibility in the return to the farms of cans that have been so cleaned and sterilized that they do not contain excessive numbers of thermoduric organisms.

Significance of thermoduric organisms. Thermoduric organisms in milk have received much attention in connection with excessive bacterial counts on pasteurized milk. In certain cases the high counts are due to inadequate heating or recontamination following heating, but in other cases they definitely are caused by relatively large numbers of heat-resistant organisms present in the original milk or added in the plant. In general, these types are of no public health significance and their resistance to heat definitely differentiates them from the pathogens that occasionally are spread through milk. Their presence in excessive numbers indicates contamination from one or more sources that include thermoduric types; such contamination can be controlled by attention to details in the procedures on the farms and in the plant.

Thermophilic organisms. Certain thermophilic organisms are capable of growth at pasteurizing temperatures. When samples of raw milk are held at such temperatures, some of them undergo changes due to the activity of organisms, whereas others do not. For the most part, the organisms which develop at pasteurizing temperatures do so only slowly; however, there are certain species that develop rapidly.

Ayers and Johnson [69] isolated a thermophilic organism from pasteurized milk that they named *Lactobacillus thermophilus.* It grows rapidly at 65.6 C (150 F) but not so rapidly at 62.8 C (145 F) or 50 C (122 F); at 30 C (86 F) growth is very slow. The organism is destroyed by heating to 71.1 C (160 F) for 30 min or 82.2 C (180 F) for 2.5 min. Hammer and Hussong [70] reported the isolation of an organism which they designated *Bacillus calidolactis* that caused coagulation of skim milk at approximately 71 C (159.8 F) under commercial conditions. The milk was intended for drying and was held hot after pasteurization. The organism does not grow below 45 C (113 F) and thus is rather strictly thermophilic. Rudig [71] reported the isolation of a thermophilic organism that withstood 76.7 C (170 F) for 20 min but not for 30 min.

It appears that thermophilic organisms sometimes develop in operating pasteurization equipment. Presumably, this occurs most frequently when milkstone is allowed to accumulate, since this prevents the organisms from being washed out of the equipment by the flow of milk. In some instances high bacterial counts on pasteurized milk that were attributed to growth of thermophilic organisms actually have been due to thermoduric rather than thermophilic types. For the most part, the thermophilic organisms encountered in milk are spore formers.

Hansen [72] fed 11 cultures (9 species) of thermophilic bacteria grown in sterile skim milk to guinea pigs; all the cultures were spore formers. The animals increased normally in weight and none of the organisms produced any detectable toxic substance. Hansen concluded that the presence of thermophilic bacteria in market milk appears unimportant from the public health viewpoint.

Comparative bacterial counts after HTST and LTLT pasteurization. Most investigations indicate that, on the average, the destruction of organisms in milk is somewhat less with HTST than with LTLT pasteurization. Differences in destruction vary widely from sample to sample and sometimes bacterial counts actually are lower with the HTST method.

In a comparison under commercial conditions of 71.1 C (160 F) for 15 to 20 sec and 61.7 C (143 F) for 30 min on milk from the same lots, Quinn and Burgwald [73] obtained the following average values, for six runs:

COMPARATIVE NUMBERS AND COMPARATIVE DESTRUCTION OF BACTERIA WITH HTST AND LTLT PASTEURIZATION

Milk	Bacteria per Milliliter	Per Cent Reduction in Count
Raw	1,066,667	
HTST pasteurized	50,271	95.28
LTLT pasteurized	35,087	96.71

In laboratory tests, holding milk at 71.1 or 72.2 C (160 or 162 F) for 25 to 60 sec did not increase the bacterial efficiency of the process; the creaming ability was decreased.

With 15 samples of milk, Hileman *et al.*[74] obtained average counts after pasteurization as follows: 61.7 C for 35 min in the laboratory, 13,000 per milliliter; 71.7 C (161 F) for 16 sec in the laboratory, 17,533; 71.7 C for 16 sec in the plant, 30,200. In the trials of Hileman and Leber [75] on 125 samples of milk, average counts on the pasteurized milk were as follows: 61.7 C for 35 min in the laboratory, 11,168 per milliliter; 71.7 C for 16 sec in the laboratory, 13,761; 71.7 C for 16 sec in the plant, 25,932.

Millenky and Brueckner [76] found that milk must be pasteurized at approximately 73.9 C (165 F) in an electric HTST pasteurizer to equal the bacterial destruction obtained with the LTLT method. When the flora was not particularly resistant, temperatures below 73.9 C resulted in a satisfactory bacterial destruction.

The following results were reported by Fay: [77] Of 262 lots of milk from 4,000-gal storage vats, pasteurized simultaneously by the HTST method at 72.2 C for 16 sec and by the LTLT method at 61.7 C for 33 min, 55 per cent gave higher plate counts after the HTST process. Of

40 samples giving essentially the same counts after the two methods, the tendency was toward a higher count after the HTST process. Results were similar for (1) milk having low initial counts of heat-resistant organisms, (2) milk having high initial counts of heat-resistant organisms, and (3) milk having high total counts (over 1 million per milliliter).

Influence of the method of pasteurization on the morphology of the surviving organisms and their reactions in litmus milk is shown by the following data of Hileman *et al:*[74]

INFLUENCE OF THE METHOD OF PASTEURIZATION ON MORPHOLOGY AND ON REACTION IN MILK OF SURVIVING BACTERIA

Character of Surviving Organisms	Laboratory Pasteurization				Plant Pasteurization	
	61.7 C for 35 min		*71.7 C for 16 sec*		*71.7 C for 16 sec*	
	No.	%	*No.*	%	*No.*	%
Morphology						
Streptococci	134	68.7	78	29.7	80	17.7
Micrococci	46	23.6	156	59.3	346	76.4
Sarcinae	8	4.1	4	1.5	5	1.1
Rods	7	3.6	25	9.5	22	4.8
Total	195	100.0	263	100.0	453	100.0
Reaction in litmus milk						
Acid	182	93.3	173	65.8	205	45.3
Alkali	8	4.1	81	30.8	231	51.0
No change	4	2.1	6	2.3	11	2.4
Peptonization	1	0.5	3	1.1	6	1.3
Total	195	100.0	263	100.0	453	100.0

Hileman *et al.*[74] concluded that the higher bacterial counts obtained with HTST pasteurization than with the LTLT method are due largely to the ability of certain *Micrococcus* species to survive the former procedure in larger numbers; the more common *Micrococcus* species found in milk pasteurized with the HTST method were *M. candidus, M. epidermidis, M. luteus,* and *M. varians.*

Comparative growth of bacteria in raw and pasteurized milk or cream. Pasteurization of milk or cream tends to kill the types of bacteria which grow most readily at relatively low temperatures. Accordingly, at such temperatures multiplication of bacteria is more easily controlled in pasteurized than in raw products, particularly if the latter have been carelessly produced. The increased period that pasteurized milk will remain satisfactory cannot be predicted because of various influencing factors but at the temperatures used for holding milk it is very substantial.

Powell [78] collected raw cream and flash pasteurized small portions of it in a laboratory apparatus at 68.3, 73.9, 79.4, and 85 C (155, 165, 175, and 185 F), after which they were held at 1.7 C (35 F) for 10 days. In the 12 trials, reduction in bacteria during pasteurization increased with an increase in temperature. The raw cream showed an increase in count during holding at 1.7 C for 10 days, whereas counts on the pasteurized portions remained essentially as they were originally. In the fresh raw cream acid-coagulating bacteria predominated but as the cream aged they were gradually replaced by the slower-growing peptonizers and acid formers. In the pasteurized cream the percentage of bacteria that were peptonizers increased as the heating temperature was raised and these organisms predominated at 73.9 C and above.

Sherman *et al.*[79] noted that at 0 C (32 F) bacterial growth in pasteurized milk was much slower than in raw milk. Uncontaminated pasteurized milk kept two to three times as long as raw milk of substantially the same quality or bacterial count. Good-quality raw milk usually kept about 4 weeks before spoilage, whereas a comparable quality of pasteurized milk usually kept in excess of 8 weeks. Inoculation of pasteurized milk with a minute amount of raw milk decreased its keeping qualities to a point substantially the same as that of raw milk. In the trials of Nicholas and Anderson,[80] pasteurized or homogenized pasteurized milk with an initial bacterial count averaging from 1,000 to 94,000 per milliliter kept in an electric refrigerator at an average temperature of 4.4 C (40 F) for an average period of 17.2 days before detectable spoilage occurred. Two batches of raw milk stored under the same conditions remained unspoiled 4 and 7 days, respectively. An oxidized flavor sometimes developed in pasteurized milk in 2 to 6 days.

Dahlberg [81] found that, in pasteurized milk held at refrigeration temperatures, the coliform bacteria increased more rapidly than the total bacterial count. In the freshly pasteurized milk <0.02 per cent of the total bacteria were coliform types. After 4 days at 1.7 to 4.4 C (35 to 40 F) in October the percentage of the total bacteria that were coliform types did not increase, but in July and August it increased to 1.12 per cent. After 4 days at 7.2 to 10 C (45 to 50 F) and at 12.8 to 15.6 C (55 to 60 F) in October the coliform organisms constituted about 5 per cent of the total count; in July and August at 7.2 to 10 C they constituted 88 per cent and at 12.8 to 15.6 C they constituted 50 per cent. Dahlberg suggested that the coliform bacteria may grow relatively rapidly in pasteurized milk because they are contaminants that have not been subjected to the heat treatments and are actively growing organisms, especially in warm weather.

Fermentation in pasteurized milk. Although bacterial growth in pasteurized milk is slower than in raw milk, the activity of bacteria eventually results in changes in the product. The fermentation that takes place shows variations, just as does the fermentation in raw milk. It is influenced by such factors as contamination subsequent to the heating, holding temperature, original flora, etc. In general, coagulation occurs and it may be caused by sweet curdling or acid formation. Coagulation due to acid formation is not noted so regularly as with raw milk and, when it takes place, microscopic examinations or plate cultures often show a variety of organisms, instead of the predominance of streptococci that is so common when raw milk develops acid. Although large numbers of acid-forming organisms may be present in pasteurized milk, at relatively low temperatures their action is slower than the action of the acid formers in raw milk, and a rapid increase in acid does not occur unless there has been significant contamination subsequent to the heating. The acid-forming streptococci that survive pasteurization commonly are types requiring relatively high temperatures for their active development. Thurston and Olson [82] reported that the pasteurization of low-count milk at 62.8 C (145 F) for 30 min changed the flora so that on storage acid developed slowly and coagulation occurred at low acidities, apparently because of the production of a rennin-like enzyme by the bacteria.

Following coagulation of pasteurized milk due to production of a rennin-like enzyme or acid, other changes occur. There may be active digestion of the milk with formation of objectionable odors; this may be due to continued activity of the organisms involved in the sweet curdling. Production of gas is common; spore-forming anaerobes may be involved although in some cases coliform types that resisted the pasteurization, or gained entrance to the milk following the heat treatment, are responsible.

At one time there was a tendency to assume that fermentations in pasteurized and raw milk are essentially the same. This probably was the result of using inadequate pasteurization exposures and permitting extensive contamination of the heated milk. The relatively rapid production of acid that is so common when raw milk undergoes bacterial changes greatly limits the growth of acid-sensitive species. It does not occur with normally pasteurized milk and other types of organisms have a greater opportunity for growth. Many consumers have complained that pasteurized milk does not sour but shows objectionable changes.

Although acid formation in pasteurized milk is relatively slow, organisms producing acid in milk, either with or without coagulation, are prominent among those surviving pasteurization. In their early studies Ayers and Johnson [83] noted that the percentage of the total flora made up of the two groups was greater in milk pasteurized at 62.8, 71.1, or 76.7 C (145, 160, or 170 F) for 30 min than in raw milk; at still higher exposures the acid-coagulating group disappeared and the acid-non-coagulating group was greatly reduced in importance. Thurston and Olson [82] studied milk of low bacterial content and noted a decrease in the acid-coagulating species from 49.87 to 18.85 per cent of the total flora as a result of pasteurization at 62.8 C for 30 min, whereas there was an increase from 18.31 to 48.01 per cent in the acid-non-coagulating organisms; changes in the other groups were much less significant.

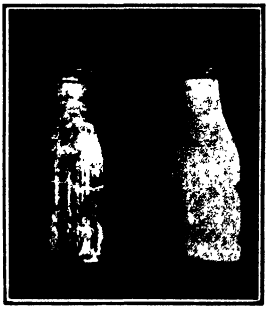

Fig. 22. Types of curd, highly heated milk (left) and raw milk (right), after 2 days at room temperature.

Advantages of pasteurization. The advantages of proper pasteurization are (1) reduction in spread of pathogens through milk, and (2) improvement in keeping qualities of milk.

1. Reduction in spread of pathogens. Exposures used in pasteurization have been selected so that pathogens present in milk will be destroyed. The process is no guarantee against future infection, however, and various epidemics have been due to contamination of milk after heating. In 1923, Godfrey [84] concluded from a study of a large number of milk-borne epidemics that 85 to 90 per cent were due to farm infection. At present, with the much more extensive use of pasteurization, farm infection of milk with pathogens is largely concealed. When properly carried out, pasteurization establishes the safety of milk at the time of heating and infection subsequent to this must be guarded against by proper equipment and satisfactory methods of handling.

The actual decrease in milk-borne diseases as a result of pasteurization cannot be determined because other factors have influenced the same diseases; for example, the decrease in typhoid fever during the last several decades has been pronounced and pasteurization of milk has played a part in this, but such factors as better raw-milk supplies, improvements in water supplies, suitable sewage disposal, immunization, etc., also have been operative, and it is impossible to ascertain the influence of these separately. The value of pasteurization is shown directly by instances in which raw milk from a certain source has caused an epidemic, and pasteurized milk from the same source has not.

Pasteurization often is begun as soon as a raw milk supply is under suspicion and various epidemics have been stopped by this procedure. Evidence of the value of pasteurization also is provided by the definite decrease in milk-borne diseases that has occurred in certain cities as a result of compulsory pasteurization; often such pasteurization has followed a severe milk-borne outbreak of a disease. In some instances cities which had experienced milk-borne epidemics more or less regularly over an extended period suddenly have become free of them following practically complete pasteurization of the milk supply. Milk-borne outbreaks have been especially numerous in countries such as the United States, where consumption of raw milk has been common.

2. *Improvement in keeping qualities.* The necessity of delivering milk with reasonably good keeping qualities is appreciated by all milk dealers. A product that readily sours or undergoes other changes is certain to bring complaints from consumers and probably will cause loss of patronage. Milk should have keeping qualities that will enable it to remain in a satisfactory condition with suitable holding for at least 3 days following delivery, so that it will be consumed before spoilage occurs. The every-other-day delivery of milk, which became common in the United States during World War II, emphasized the need for good keeping qualities in milk and showed that they are easily attainable.

Pasteurization is extremely valuable in giving milk satisfactory keeping qualities. In some cities the consumers are so far from the producing farms that the milk is 1 to 2 days old when it is delivered, and, if it is to keep at least 3 additional days at the usual holding temperatures, some such treatment as pasteurization is necessary. Even in small towns, where the milk is delivered at an age not exceeding 12 to 18 hr, the keeping qualities may cause annoyance during the warm months unless pasteurization is employed. The difficulty is increased when the dealer does not have complete control over the production and so cannot insist on methods that will give a low bacterial count. The danger of some unusual contamination suddenly giving milk poor keeping qualities, and thus causing complaints, has forced many dealers to employ pasteurization for their own protection. With the success of a business depending on the satisfaction given by the product supplied, every safeguard is advisable and proper pasteurization provides a valuable form of insurance. The primary object of pasteurization should be to provide safe milk, but the other advantage of the process need not be ignored. With customers justifiably demanding milk that keeps well and with a complex milk-distribution system over which the dealer does not always have complete control, use of pasteurization to improve the keeping qualities is advisable.

Objections that have been raised to pasteurization. From time to time since the introduction of pasteurization, various objections to the process have been raised. Often these have come from persons who prefer to sell milk in a raw condition, because of the expense of installing pasteurizing equipment, and attempts to establish regulations requiring pasteurization sometimes result in protests, especially from distributors whose supplies are so small that expenditures for equipment must be kept very low. Objections to pasteurization also have come from physicians and others who prescribe the type of milk to be used by infants and invalids, and from consumers.

At one time there may have been some foundation for certain of the objections but improvements in pasteurization methods, in quality of the raw milk, and in the general understanding of the process have removed any basis for most of them, and objections now come largely from persons who do not appreciate what the process accomplishes.

Objections to pasteurization which have been most frequently advanced are:

1. *Organisms developing in pasteurized milk form harmful products.* This idea appears to have been based on the work of Flügge,[85] who produced illness in puppies by feeding them milk cultures of organisms obtained from boiled milk. The results are not directly applicable to pasteurized milk, since the organisms were isolated from milk heated to much higher temperatures than those used in pasteurization, but the species which survive boiling also would survive pasteurization. Development of toxic products by either pure or mixed cultures in milk held for extended periods is quite different from such development in milk held under the usual conditions. With long holding at temperatures favorable for bacterial growth, either raw or pasteurized milk may contain products that are toxic for experimental animals, but ordinarily the milk has undergone conspicuous changes and would not be used for food.

2. *Infants do not develop so well on pasteurized as on raw milk.* Occasionally, the claim is made that pasteurized milk is not satisfactory for infants. The best argument against this is the large number of

well-developed children that have been fed the product. Careful observations in various cities show that many infants make entirely normal gains on pasteurized milk.

Convincing evidence of the value of pasteurized milk for children was obtained by Frank *et al.*[86] in a field study. Because practically all the children had received some heated milk, the raw-milk group included those children who had received raw milk for more than half their lives, including the latter half, whereas the heated-milk group included those children who had received heated milk for more than half their lives, including the latter half. Results on more than 3,700 children from 10 mo to 6 yr of age showed that there was no significant difference between the average weights or heights of the two groups. However, parents of children receiving predominantly raw milk reported a higher incidence of diphtheria, scarlet fever, intestinal disturbance, and rickets than did parents of children receiving predominantly heated milk.

3. Products of bacterial growth are not destroyed. It is probable that certain toxic products formed by extensive growth of bacteria in milk resist pasteurization exposures, but if the same milk were consumed in a raw state the toxic products would be equally, or more, active. Since these products are not the result of pasteurization but develop before it is used, the process cannot be held accountable for them. Milk in which toxic products are present as the result of bacterial action must contain large numbers of organisms and these indicate unsatisfactory methods of production or handling; such milk is not satisfactory for consumption either raw or pasteurized.

4. Pasteurization may be used to mask dirty milk. In some instances pasteurization undoubtedly has been employed to give milk which has undergone extensive bacterial growth a keeping quality that made it marketable. However, milk that is to be pasteurized requires the same supervision as milk that is to be sold raw, and pasteurization cannot replace unsatisfactory methods of production and holding. With adequate standards for milk coming to pasteurizing plants, producers soon appreciate that suitable methods are necessary. Improper use of pasteurization cannot be blamed on the process and can be stopped through supervision of the supplies intended for heating.

5. Milk enzymes are destroyed. The normal milk enzymes are partially or completely destroyed by the usual pasteurization exposures. However, there is no evidence that these enzymes play a part in the digestion process and any influence that pasteurization has on them probably is unimportant from the standpoint of the person consuming the milk. Destruction of lipase by pasteurization is a distinct advantage with certain lots of milk that would become rancid if left in a raw state.

6. *Influences the composition of milk.* Investigations indicate that pasteurization at the usual exposures has little effect on the composition of milk. Rupp[87] made a series of analyses on milk samples pasteurized at various temperatures for 30-min periods. Milk heated to 62.8 C (145 F) did not undergo an appreciable change. The soluble phosphates of lime and magnesia did not become insoluble. At 68.3 C (155 F) the quantities of phosphoric acid, lime, and magnesia in the serums of raw and pasteurized milk were practically the same. The albumin did not coagulate at 62.8 C, but at 65.6 C (150 F) 5.75 per cent was rendered insoluble, and as the temperature was increased the amount of coagulated albumin increased. The period required for coagulation by rennin was slightly less in milk pasteurized at temperatures up to 65 C (149 F) than in raw milk, but at 70 C (158 F) it was slightly increased and at 75 C (167 F) it was almost doubled.

Elvehjem[88] reported that from work with dogs there was no indication of a reduced availability of calcium and phosphorus in pasteurized milk; blood calcium and phosphorus were determined and there was no difference in the blood pictures of the dogs on the different types of milk.

Effect of pasteurization on the physical condition of fat in milk is definitely objectionable from the standpoint of the dealer when it results in a significantly decreased cream line.

7. *Influences vitamins.* With an appreciation of the value of the vitamins in milk, the question of the influence of pasteurization on them became of importance. It is now recognized that the type of pasteurization, condition of equipment, etc., greatly influence the results. There are differences in the vitamin contents of raw milk as a result of season, feed, condition of the animal, and method of handling, and these may be of more significance than the differences due to pasteurization.

Elvehjem[88] noted that pasteurization does not lower the vitamin A or carotene content of milk and there is no indication of loss of vitamin D, but that there may be some destruction of the other fat soluble vitamins, E and K.

Woessner *et al.*[89] found that pasteurization by the LTLT method caused a 20 per cent loss of ascorbic acid. They noted that the advantage of HTST pasteurization in preserving ascorbic acid seems dependent upon the fact that the destruction of ascorbic acid appears to be related more to the period it is held at the elevated temperature than to the temperature itself. The most serious losses in antiscorbutic activity during processing and delivery of milk were caused by copper contamination and exposure to light.

At irregular intervals during 18 mo, Holmes *et al.*[90] pasteurized 20 lots of milk with the HTST process. The average values obtained just before and immediately after pasteurization were: for ascorbic acid, 16.4 and 16.6 mg per liter, respectively; for riboflavin, 1.50 and 1.50 mg per liter, and for thiamin, 0.36 and 0.35 mg per liter.

Elvehjem [88] noted that vitamin B_6 and nicotinic acid are very stable and no loss of these factors on pasteurization would be expected. Also, that preliminary results indicate pasteurization does not inactivate pantothenic acid.

With LTLT pasteurization in a stainless steel vat, Holmes *et al.*[91] obtained the following effects on vitamins: ascorbic acid, raw milk (32 samples) 19.7 mg per liter, pasteurized milk (32 samples) 15.9 mg; riboflavin, raw milk (31 samples) 1.51 mg per liter, pasteurized milk (31 samples) 1.48 mg; thiamin, raw milk (29 samples) 0.33 mg per liter, pasteurized milk (31 samples) 0.30 mg. Holmes [92] reported that pasteurization by the LTLT and the HTST processes showed the following effects on riboflavin: 24 lots LTLT pasteurization (stainless steel, spray vat), 1.46 mg per liter before and 1.43 after; 12 lots LTLT pasteurization (stainless steel, coil vat), 1.52 mg per liter before and 1.49 after; 10 lots HTST pasteurization, 72.2 to 82.8 C (162 to 181 F) for 15 sec (depending on directions of manufacturer), 1.41 mg per liter before and 1.42 after.

Theophilus and Stamberg [93] reported that neither pasteurization, homogenization, nor storage for 24 hr at 4.4 C (40 F) in a dark refrigerator decreased the riboflavin content of milk. As much as 40 per cent of the riboflavin in milk was destroyed by 2 hr exposure to direct sunlight in clear glass bottles even though the milk was quite cool; good shade, brown glass bottles, or paper containers gave very good protection.[94]

Special temperature and time relationship. The shift from LTLT to HTST pasteurization, with the general advantages and with no serious disadvantages, suggests the possibility of an additional increase in temperature and corresponding decrease in time. Holland and Dahlberg [95] reported that a point was found at 76.7 C (170 F) that appeared especially promising as a new standard for pasteurization. Later, Dahlberg *et al.*[96] conducted experiments under commercial conditions in which the heat treatment given the milk consisted of a controlled period above 60 C (140 F) during which the milk was heated to the desired temperature and again cooled below 60 C; there was no definite holding at the highest temperature attained. The method was designated quick-time pasteurization. Milk was successfully quick-time pasteurized at 80.8 to 76.1 C (177.5 to 169 F), with the period above 60 C varying from 5 to 24 sec. Milk was pasteurized at 76.6 C with 12 sec as the total period to heat and to cool. At the higher temperatures the variation in the highest temperature attained could be greater than the present accepted standards and still show proper pasteurization on the basis of the phosphatase test and destruction of *E. coli.* Data were not obtained on pathogenic organisms.

Increase in use of pasteurization. When pasteurization was introduced into the milk industry the main object was to improve the keeping qualities of the product and, accordingly, the process was sometimes used surreptitiously. However, a clear understanding of what pasteurization accomplishes eventually established confidence in it. As a result of the epidemics traced to dairy products and the finding of pathogens, especially *M. tuberculosis,* in these materials, certain agencies supervising milk supplies began to require pasteurization of all milk, except that produced under special conditions. There also has been a great increase in the use of the process in cities where it is not compulsory because its advantages have come to be appreciated by milk dealers. When a milk-borne outbreak of a disease occurs there often is an immediate demand for pasteurization in the locality involved. Many persons interested in the safety of the foods they consume insist on pasteurized milk and this demand is being stimulated, in certain instances, by advertising and educational campaigns that point out the value of pasteurization. Pasteurized milk is no longer confined to the larger cities and it now constitutes a considerable percentage of the supplies of many smaller communities. The public generally is beginning to appreciate the danger of spreading diseases through raw milk and to recognize the protection given by adequate pasteurization.

In some instances attempts have been made to satisfy a demand for milk pasteurization by advocating the testing of the producing cows for various diseases. Although diseased cows are highly objectionable, they are only one of the sources from which pathogens enter milk. Proper pasteurization kills all the usual pathogens that are present in milk at the time of heating regardless of their source and, accordingly, is more inclusive than any system of cattle testing. Regulations which require either pasteurization or cattle testing do not take into account the difference in accomplishments of the two procedures.

Tests for pasteurized milk. Various tests have been used in attempts to determine whether pasteurization and/or subsequent handling of milk have been carried out satisfactorily.

At one time low plate counts on pasteurized milk often were interpreted as indicating adequate methods. However, low plate counts can be obtained on underpasteurized milk due to low-count raw milk or to a flora having little heat resistance. On the other hand high plate counts are obtained with adequate heat treatment because of a heat-resistant flora in the raw milk; contamination following pasteurization and growth of organisms in the pasteurized product are other possible causes.

Presence of coliform organisms in pasteurized milk sometimes has been interpreted as indicating inadequate heating or recontamination. However, there are coliform strains that resist the usual pasteurization exposures so that a coliform culture obtained from milk should be tested for heat resistance to establish that this is not relatively high. Also, growth of coliform organisms at relatively low temperatures appears to be rather common and high coliform counts on milk that has been held may be the result of very limited contamination, or survival, and growth, rather than more extensive contamination.

Following tests with other milk enzymes, especially amylase, the presence of more than a very limited phosphatase activity in milk is now accepted as indicating underpasteurization. Such inadequate heat treatment may be due to a low temperature and/or a short exposure; also, it may be due to contamination of properly pasteurized milk with raw milk or an underheated portion. The phosphatase test is the basis for adequate control of the pasteurization process. Although certain bacteria produce phosphatase, under practical conditions they are of little importance as a cause of positive phosphatase tests on milk. The phosphatase test cannot detect recontamination of pasteurized milk or growth of bacteria in it and these still must be controlled by total bacterial counts or counts of some special type of organism.

ELECTRICAL TREATMENT OF MILK

Studies concerning the effect of an electric current on bacteria have included attempts to destroy most of the organisms in milk by passing a current through it. In all the investigations the important point has been whether the effect on the organisms is due to the current or the heat generated by it.

Beattie and Lewis [97] found that milk could be rendered free of *E. coli* and *M. tuberculosis* by an electrical method without raising the temperature higher than 63 to 64 C (145.4 to 147.2 F). The temperature effect was very short and in itself was not the principal factor in the destruction of bacteria. The keeping property of the milk was increased and the taste was not altered. Later, the investigators [98] again concluded that the heat is not the main factor in the destruction, but that the electric current is an important agent. With the method of treatment used, bacteria were destroyed at temperatures much below their thermal death points.

Anderson and Finkelstein [99] studied a process for electrical treatment of milk in which the milk was heated with steam to 40 C (104 F), delivered to a supply tank, and then run to the machine itself, which was made up of units each consisting of five porcelain cups so arranged that the milk flowed from one to the other. The first cup had about twice the capacity of the others. In each of the four small cups there was an electrode, which was an insulated rod terminating in a copper disc. The current was completed by the stream of milk. The voltage was 2,300, the amperage 14, and the frequency 25. The milk was raised to about 70 C (158 F) in the machine and then went to a cooler. The method gave very satisfactory destruction of bacteria with good-quality milk and satisfactory destruction with poor-quality milk; the treated milk kept well. Precipitation of albumin was slight, if it occurred at all, and the cream line was not influenced. The destruction of bacteria appeared to be due to the heat produced.

Prescott [100] investigated an installation for treating milk in which the milk was heated to about 47.8 C (118 F) in a regenerative cooler and then subjected to an alternating current of 220 volts, 60 cycles. In the treating chamber the milk was raised to 70 to 71.1 C (158 to 160 F) and was then cooled to a low temperature. The milk did not have a cooked taste, the cream line was well marked, and the keeping quality was excellent. During the winter, when the raw milk had a count of <200,000 bacteria per milliliter, the percentage reduction in the organisms approximated 90 per cent; in the warmer months, with the raw milk running from 500,000 to 600,000 per milliliter, the reduction varied from 97 to 98.5 per cent. No *Escherichia-Aerobacter* organisms were found in the treated milk. Under the recommended commercial conditions of 71.1 C, 220 volts, 60 cycles, *M. tuberculosis* was destroyed.

In laboratory trials Gelpi and Devereux [101] found a much lower destruction of bacterial spores added to milk when pasteurizing at 62.8 C (145 F) for 30 min than when subjecting the milk to an electrical treatment in which 35 to 40 sec were required to pass through the machine, 10 to 14 sec being the period the temperature was at 71 C (159.8 F). With the various species the percentage reductions with pasteurization were 0 to 13.0 per cent, whereas with the electrical treatment they were 71 to 99.9 per cent. In further studies the investigators [102] obtained results which indicated that destruction of endospores by an electrical process is not entirely due to the heat created in the medium surrounding the spores but also to another heat factor, namely, the heat generated within the spores. The temperature attained within the spores probably is greater than that of the surrounding medium.

Tracy [103] passed an alternating current through yeast cell suspensions in grape juice at non-lethal temperatures—42 C (107.6 F)—and obtained pronounced killing effects, indicating that an alternating current of 60 cycles exerted a lethal effect independent of temperature. The lethal effect varied with current density and quantity of electricity used. He considered that the killing effect of the current may be caused by formation of temporary toxic substances that are immediately reduced on cessation of the current and thus disappear.

EFFECT OF ULTRA-VIOLET RAYS ON BACTERIA IN MILK

The attempts that have been made to destroy the organisms in milk have included trials with ultra-violet rays.

Houghton and Davis [104] investigated the action of ultra-violet rays on bacteria in various materials, when these flowed through a trough exposed to a lamp. With milk, a comparatively low reduction in the organisms was obtained. The ultra-violet rays had a tendency to kill the undesirable organisms present, leaving *S. lactis* in the majority. There was no perceptible change in the taste of the milk as a result of the exposure.

Ayers and Johnson [105] made a series of tests in which milk was exposed in various ways to ultra-violet rays generated by a quartz mercury-vapor lamp operating on a 220-volt circuit and taking 3.5 amperes. The most satisfactory method of exposure was over two revolving drums, the tops of which were 4 in. below the light tube. The milk picked up by one drum was exposed, then collected by a scraper, after which it was picked up by the second drum and again exposed; finally, it was collected by a second scraper. When the milk was exposed in thin layers there was a marked reduction in bacteria and this was independent of the action of the heat, since the temperature of the exposed milk never was above 30 C (86 F). The two most important factors in the success of the method were thickness of the layer of milk and length of exposure. Results on two samples of exposed milk indicated that the rays did not have a selective action on organisms, although there was a greater effect on vegetative cells than on spores. When milk was exposed under conditions suitable for satisfactory reduction of bacteria by the rays, an abnormal, disagreeable flavor was produced. Under similar conditions of exposure there appeared to be less reduction of organisms in 15 per cent cream than in milk, and this was considered to be due to the thicker layer in which the cream was carried by the drums.

Weckel [106] reported that radiation of whole milk of high quality under commercial conditions appears to have little influence on the bacterial counts, but that with milk of poor quality the counts are reduced. Radiation did not have a selective action on organisms occurring naturally in milk, or on such species as *S. lactis, E. coli,* or *B. coagulans* when introduced into the milk.

Supplee *et al.*[107] noted that the lethal effectiveness of ultra-violet energy applied to smooth-flowing milk films is dependent upon intensity and special characteristics of the radiation employed. A greater bactericidal effect was obtained with ultra-violet radiation applied to smooth-flowing milk films than to films flowing over a corrugated cooler surface. Percentage reduction in bacterial counts on raw mixed milk in excess of 90 per cent and, frequently, up to 95 to 98 per cent may be obtained with a high degree of regularity by preferred methods of applying ultra-violet radiation of appropriate intensity and spectral characteristics.

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Chapter 10

Bacteriology of Evaporated Milk

Large volumes of milk are converted to evaporated milk in order to supply areas in which fresh milk is not readily available and also to provide milk that can be stored on the same basis as many other foods. The keeping properties depend on destruction of the contained organisms during the sterilization process and, when this actually occurs, there is no spoilage due to micro-organisms. However, bacteria occasionally survive the heat treatment and some of them are species that can cause abnormal conditions in the product. The water and nutrients in evaporated milk make it well suited to growth of many types of organisms and, since nothing is added to control growth, changes often take place when the heat treatment is incomplete. In general, organisms which survive the heat treatment are spore-forming bacteria that are relatively unimportant in other dairy products so that evaporated milk presents a more or less characteristic bacteriological problem.

Occasionally, a can of evaporated milk spoils as a result of the entrance of organisms through a defect in the tin. Under these conditions non-spore-forming bacteria commonly are involved.

Bacteriological examination. In the bacteriological examination of a can of evaporated milk, great care is needed in opening in order to avoid contamination. First, the can should be washed thoroughly and dried with a clean cloth. Then, a small area can be flamed, it can be covered with alcohol and the alcohol burned, or it can be flooded with solder. The can is punctured in the treated area with a freshly flamed piece of metal, such as a nail. Material is taken from the can with a sterile glass tube drawn to a fine bore so the puncture can be kept small. It can be inoculated into milk or any desired liquid medium, or smeared on agar, and incubated aerobically and/or anaerobically. Plate counts can be made if desired but, ordinarily, they are of no particular value. Often it is desirable to reseal the can for further tests and this is easily done by soldering if an area on the can was flooded with solder.

Material from a can of evaporated milk can be stained and examined microscopically; ordinarily, spoilage organisms are readily seen. With certain stains and careful observations, a portion of the organisms that were killed by the heat treatment can still be seen; normally, these are not confused with active spoilage types because of their poor staining properties. Leucocytes in evaporated milk also are readily seen in stained preparations.

Normal evaporated milk. When cultured in milk or on agar, normal evaporated milk commonly does not yield organisms. Weinzirl[1] found no living organisms in 23 cans. Deming and Davis[2] studied 154 cans, using anaerobic and aerobic cultures at 37 C (98.6 F) with 102 of them and anaerobic and aerobic cultures at 55 C (131 F) with 104. No growth was obtained except in one anaerobic culture at 55 C which showed a spore-forming rod; attempts to perpetuate the culture were unsuccessful. Nichols[3] found 35 cans of evaporated milk and 17 cans of canned cream from general sources were sterile. In the laboratories maintained by certain evaporating plants, cultures prepared in checking the sterilization efficiencies commonly are negative.

Occasionally, evaporated milk that is normal in appearance readily yields bacteria on culture. Certain of them, although developing large colonies on agar, produce little if any change in milk and it appears that milk is an unsatisfactory medium for them; organisms with these general characters have been found for extended periods in the product of a plant without causing defects. Other organisms in evaporated milk apparently are prevented from growing, or are greatly limited in their growth, by lack of air; when air is provided growth is rapid and conspicuous changes occur. Still others have such high growth temperatures that they do not develop under the usual holding conditions for evaporated milk. In some instances normal-appearing evaporated milk has contained surprisingly large numbers of bacteria per milliliter.

Theophilus and Hammer[4] found 1 of 15 cans (7 brands) of evaporated milk yielded colonies on culture, all of which appeared to be of one species; odor and flavor of the milk were normal. Spores of the organism were rather easily destroyed by heat. Nichols[3] cultured evaporated milk and canned cream from selected plants. With 235 cans of milk and 52 cans of cream from one factory, 94 and 85 per cent, respectively, were sterile; in another factory the percentages were 93 (with 44 cans) and 100 (with 6 cans); for still another factory they were 100 (with 1 can) and 76 (with 178 cans). It appeared the comparatively low sterility percentages for some samples were related to special circumstances of operation. In one factory more than one-half the un sterile cans were produced during a 10-day period when processing apparently was not carried out so efficiently as usual. Samples examined by Nichols because they were obviously defective, or associated with defective packs, showed a much higher percentage of unsterile cans.

Sterilization exposures. With evaporated milk the proper sterilization exposure is very important. The heating must not be too severe because of the undesirable chemical changes that result, but it should destroy all the bacteria present. No general statement can be made as to the exposure required since it varies with a number of factors, and an exposure that is entirely satisfactory under one set of conditions may not be under another. Because of the development of some unusual situation, it sometimes is necessary to abandon an exposure that has been satisfactory over long periods; most often the exposure is increased in an attempt to destroy some very resistant organism that suddenly has caused spoilage.

Each evaporated milk plant has developed a sterilization procedure, either batch or continuous, which appears to be satisfactory for its conditions. The exposures used commonly are varied slightly with the different sizes of cans and sometimes with the quality of the original milk. A plant may use various procedures that it considers important in carrying out the sterilization. In some instances these include use of relatively low heat treatments in order to limit heated flavor and darkening and thus obtain a product that is especially pleasing to consumers.

It appears that relatively high temperatures for relatively short periods offer some interesting possibilities in connection with the sterilization of evaporated milk. Such a procedure involves difficulties from the standpoints of equipment and physical changes in the product that need additional study.

Factors influencing heat resistance of spores. From the exposures used it is evident vegetative cells never survive the heat treatment of evaporated milk and only spores can cause difficulty in this way. Various factors undoubtedly influence the heat resistance of spores of a given species. Although some of the factors are of little importance in evaporated milk, because of its relatively constant composition and the uniform methods of production, others may be of significance.

In studying the influence of various factors on heat resistance of spores, Curran [5] noted that aqueous suspensions of spores subjected to long-continued freezing at −15 C (5 F) became less heat resistant, whereas alternate freezing and thawing were not so effective in reducing the resistance. Storage of spores at moderate temperatures was more conducive to enhanced resistance than storage at low or high temperatures. Prolonged action of metabolic products tended to reduce the resistance materially. Spores formed and aged in soil or in oats were much more heat resistant than those formed and aged in broth or on agar. Hastings [6] had previously found that certain bacteria remained viable much longer in their natural habitats than under artificial conditions.

Theophilus and Hammer [4] found with certain aerobic, spore-forming bacteria isolated from normal and spoiled evaporated milk and from raw milk that growth at the approximate optimum temperature commonly yielded spores with greater thermal resistance than growth below or above that temperature. Sudden decreases in growth temperature from the approximate optimum usually decreased the resistance of the spores produced and sudden increases to the approximate optimum usually increased the resistance, thus indicating the influence of growth temperature on thermal resistance. Comparisons of the resistance of moist and freshly dried spores (6 to 8 days) indicated there was no regularity in the effect of drying; growth temperature influenced the resistance of dried spores in the same manner as moist spores. Aging of dried spores (47 to 69 days) did not significantly decrease the resistance. A strain of *B. megatherium* isolated from spoiled evaporated milk showed a comparatively high thermal resistance, whereas a strain isolated from raw milk did not.

Curran and Evans [7] reported that sublethal heating of the spores of thermotolerant and thermophilic aerobes had a determining influence on the number of spores which germinated subsequently. They found in a study of 12 thermotolerant cultures isolated from 10 outbreaks of spoilage in evaporated milk that nearly all would respond to pregermination heating; in the absence of such treatment a large proportion of the potentially viable spores did not germinate. The proportion of spores which responded to preheating was dependent on amount of heat, composition of the medium in which they were heated, temperature at which the spores were formed, and temperature at which they were subcultured. The authors suggested that, in the manufacture of evaporated milk, forewarming the milk at 95 C (203 F) for 10 min prior to condensation results in the majority of the spores of thermotolerant species becoming heat labile during the period the milk is being condensed and that, as a consequence, they are easily killed by the subsequent processing; thus an established commercial practice conduces to a desired result.

In investigations on heat resistance of spores, the effect of environmental factors on the response of heated spores should be recognized. Apparently, in some cases, what appears to be variability in results actually is caused by lack of favorable conditions in the medium.[8] In trials with an organism causing spoilage in evaporated milk, regular results,[9] without dormancy or delayed germination, were obtained when evaporated milk was used as the subculture medium but not when nutrient broth was employed; in its enzymatic functions, the organism seemed peculiarly adapted to the environment supplied by milk.

Incubation. At one time, cans of evaporated milk often were incubated at the plant from one to several weeks with the idea of giving incompletely sterilized or defective cans an opportunity to spoil so that they could be detected easily. In some instances the incubation was carried out at room temperature; in others a warm room was used to favor activity of any organisms in the milk. Usually, the incubation accomplished its purpose but, occasionally, the organisms that had not been killed by the heat treatment developed so slowly spoilage was not evident until the milk had left the plant. At present, incubation of cans of evaporated milk is unusual. In some instances the freshly packed milk goes rather directly to cars or trucks for transportation to markets.

In the supervision of the product of a plant, it often is desirable to incubate a number of cans from each lot of milk at a relatively high temperature, such as 37 C (98.6 F), and then examine them organoleptically and also culturally. Although this procedure is very useful, it should be recognized that in certain outbreaks of spoilage the numbers of cans containing the causative organism are so small they are easily missed if only a few cans are studied.

SPOILAGE DUE TO HEAT-RESISTANT ORGANISMS

The more important fermentations that evaporated milk undergoes as a result of activity of heat-resistant organisms are coagulation, gas formation, and development of off flavors, especially bitterness. At one time gas formation was the most common defect but now coagulation is noted more frequently. The percentage of spoiled cans in a defective lot of evaporated milk is extremely variable, owing to differences in extent of the infection of the milk with the resistant organism and to variations in the balance between the heat resistance of the organism and the sterilization exposure.

In many cases of spoilage due to heat-resistant organisms the causative species is present in pure culture and is readily isolated when the milk is cultured under suitable conditions. Valuable information often is obtained by direct microscopic examination of the spoiled milk.

Coagulation. Various organisms have caused coagulation of evaporated milk; often the milk also has an abnormal odor and flavor.

The Vermont Agricultural Experiment Station[10] reported an instance of coagulation of evaporated milk in which the change was noted 12 to 24 hr after heating; the milk curdled without souring and then the curd apparently digested, leaving a whey-like liquid above a mass of slimy curd. *B. subtilis* was the organism most actively involved.

Hammer[11] studied an outbreak of abnormal evaporated milk in which the typical change was firm coagulation, although a very small percentage of the spoiled cans that were not included in the investigation showed a bulging. It was reasonably certain that at least some of the spoiled milk was heated to 113.3 C (236 F) for 36 min. May and June milk gave the greatest percentage of spoilage. The period required for coagulation could not be determined but the heated milk was held at the plant for at least 10 days and was not coagulated then. The spoiled milk had a sweetish, cheesy odor, not at all disagreeable, and a flavor that was faintly sour and cheesy. The acidity was higher than that of unspoiled milk from the same lot. An organism was quite regularly observed in microscopic preparations from the abnormal milk, and when it was cultivated and inoculated into normal cans of evaporated milk it produced the typical abnormal condition; it was described and named *Bacillus coagulans.* Cordes[12] investigated an outbreak of "flat-sours" in a condensery and found *B. coagulans* responsible. The cans from which the organism was obtained had been subjected to 114.4 C (238 F) for 20 min in a batch sterilizer. However, milk cultures of the organism were killed when exposed in an autoclave at 111.7 to 112.8 C (233 to 235 F) for 15 min; with this exposure the milk itself reached 112.2 C (234 F), was between 100 and 112.2 C (212 and 234 F) for 6 min, and between 107.2 and 112.2 C (225 and 234 F) for 4 min.

Sarles and Hammer[13] studied an outbreak of coagulation in evaporated milk and found it was caused by *B. coagulans.* The outbreak occurred during an abnormally hot, dry summer and extended over several weeks. The percentage of cans that spoiled varied from lot to lot and in one instance was estimated by the plant operators to be 60 per cent. At the condensery acidity of the normal milk was 0.48 per cent, calculated as lactic acid; acidity of the coagulated milk was 1.05 per cent. Odor and flavor of the spoiled milk were distinctly cheesy and acid, but were not suggestive of putrefaction. Spoilage began with lots of milk subjected to a heat treatment that had been satisfactory over a long period. With the appearance of the defect the sterilizing temperature was increased somewhat but coagulation of some of the cans continued. Plate counts on spoiled milk held for considerable periods at the condensery varied from 7,000 to 77,000 per milliliter; counts on milk soon after it had coagulated as a result of inoculating *B. coagulans* and holding at 37 C (98.6 F) were much higher.

FIG. 23. Coagulated material from a can of evaporated milk artificially inoculated with *Bacillus coagulans*. Iowa Agr. Exp. Sta. Res. Bul. 19.

Kelly[14] studied three types of spoilage in evaporated milk. In type A, a custard-like clot was formed at the top of the milk but there was no other change; in type B all the milk in a can clotted with a clean even curd and a slightly bitter, metallic flavor; in type C some gas was formed, the milk was clotted solid, and it had a cheesy odor. The responsible organisms were considered to be an atypical form of *Bacillus cereus,* an atypical form of *Bacillus simplex,* and an atypical form of *Bacillus megatherium,* respectively.

An organism that produced a non-acid, non-gaseous coagulation in cans of evaporated milk was studied by Morrison and Rettger;[9] they considered it to be a relatively stable variant of *Bacillus vulgatus.*

Bacillus calidolactis was found by Hussong and Hammer[15] to be the cause of coagulation in evaporated milk held at a relatively high temperature, for example 50 C (122 F), or above. The milk came from two condenseries. The organism produced a distinctly acid and slightly cheesy odor and flavor in evaporated milk. *B. calidolactis* probably is of little importance as a cause of spoilage in evaporated milk under practical conditions because of its thermophilic character.

An organism that coagulated evaporated milk in an unusual manner was studied by Hammer and Hussong.[16] It was found in only three of many cans examined in a study of coagulated milk returned to a condensery from various cities, and these were from one lot. The organism curdled evaporated milk slowly at 37 C (98.6 F) with no change in the odor and flavor, unless incubation was extended. It could be found microscopically for only a short time after inoculation into cans of milk and then only in small numbers. If the inoculated milk was given a good air supply by transferring it to test tubes, removing part of the milk from the can, or in some other manner, the organism curdled the milk rapidly, produced an objectionable odor and flavor, and could be found in large numbers microscopically. The organism was identified as *B. cereus*. The unusual point in connection with its action in cans of evaporated milk was the coagulation with no change in odor and flavor, unless the holding was extended. The organism was not considered the primary cause of the coagulation in the outbreak investigated since it was isolated from inoculated cans after extended incubation so that it probably had not died out in the milk failing to yield it; also, there was an increase in soluble and amino nitrogen in evaporated milk that coagulated following inoculation with the organism, whereas the soluble and amino nitrogen in the usual milk from the outbreak were the same as in normal milk. Extent of the coagulation in cans returned to the condensery varied from a few small masses of soft curd to complete coagulation. Presumably, an instability in the milk and high holding temperatures were responsible for coagulation in most of the cans.

Gas formation. Cans of evaporated milk sometimes undergo a gassy fermentation which results in bulging, particularly at the ends of the cans, and sometimes in bursting along a seam. Coagulation usually accompanies the gas formation and often objectionable odors develop. When bulged but unbroken cans are punctured, the contents may foam through the opening for considerable periods. Because of variations in the condition of the contents of gassy cans, it is evident that the causative organism is not always the same.

The spore-forming anaerobes constitute one of the important groups that produce gas in evaporated milk. These organisms are unusually heat resistant and commonly are responsible when there is a considerable percentage of blown cans in a lot of milk. Various species appear to be involved and, although most of them produce very objectionable odors, some do not. In the cases investigated by Hunziker,[17] gas formation accompanied by intense putrefaction regularly was due to *Plectridium foetidum.*

Bulging of cans of evaporated milk may result from causes other than bacterial action. The more common of these are:

1. *Chemical action.* Acid present in evaporated milk may act on the metal and combine with a part of the iron, thus liberating hydrogen. This is not likely to occur except after long periods. The action was demonstrated experimentally by Hunziker and Wright.[18] Tin cans were scratched on the inside with a file, filled with a 0.4 per cent solution of lactic or acetic acid, and sealed and sterilized; on incubating at 32.2 C (90 F) the cans began to swell, whereas the check cans containing distilled water did not. Savage and Hunwicke [19] consider it improbable that tins of evaporated milk become blown through chemical action alone.

2. *Filling cans with very cold milk.* When cans are filled with very cold milk and sealed, they may, after processing, show a bulging at ordinary temperatures due to expansion of the contents. Variations in atmospheric pressure may be a contributing factor in this type of bulging.

Ordinarily, it is easy to recognize bulging that is due to bacterial action by the odor and other changes in addition to the gas formation, and by the presence of numbers of bacterial cells in microscopic preparations.

Bitterness. Occasionally, a bitter flavor is encountered in evaporated milk. Sometimes it accompanies coagulation, as would be expected from the fact that certain organisms coagulating milk also digest it with formation of bitter products; in other cases it appears to be the conspicuous change and coagulation is not noted.

Hammer [20] investigated bitterness in evaporated milk and isolated an organism that produced the condition on inoculation. The first isolation was made from a can showing bitterness and an abnormal odor but no evident coagulation, whereas later the organism was obtained from a can of coagulated milk supplied by the plant reporting the defect. With extended holding, inoculated evaporated milk showed complete coagulation; microscopic examinations of the milk suggested that the organism had largely died out. The organism was named *Bacillus amarus.*

Spitzer and Epple [21] studied bitter evaporated milk in which the bitterness was the only objectionable feature. The condition did not appear in all the cans of a lot. The causative organism agreed morphologically and culturally with *Bacillus panis.* It rapidly produced large amounts of peptone and lower complex nitrogenous compounds that may have caused the bitterness. The organism was destroyed in 8 to 10 min at 121.1 C (250 F) under steam pressure.

Nichols *et al.*[22] isolated three organisms from defective canned cream that, on inoculation into normal cream, caused bitterness and thinning. The organisms were strains of *B. subtilis;* their spores withstood temperatures up to 120 C (248 F) for 40 min. Rate of development of bitterness and thinning in experimentally inoculated cans of cream varied with the culture and with the incubation temperature. The bitterness and thinning were associated with breakdown of the protein, as indicated by determinations of the non-protein nitrogen and the peptone and subpeptone fractions. The alteration in viscosity did not appear to be due to any fundamental change in structure of the cream nor to differences in clumping of the fat globules.

Thirty-one strains of aerobic spore formers from normal and spoiled cans of evaporated milk and cream were inoculated into normal cans of the products by Nichols; [3] the outstanding defect induced was a bitter flavor associated with thinness.

SPOILAGE DUE TO DEFECTIVE CANS

An occasional can of evaporated milk spoils as a result of a defect in the container that permitted the entrance of organisms. The defect may be present when the can is filled or it may develop during holding. The fermentations that occur under these conditions are of various types and, commonly, are due to mixed floras. Often the flora is made up largely or entirely of non-spore-forming bacteria; this indicates that the organisms did not survive the heat treatment since any attempt at sterilization would kill the non-spore-forming types. The species causing the different fermentations commonly are much the same as those causing similar fermentations in raw milk. Rarely, non-spore-forming bacteria are found in evaporated milk in pure culture, a fact which suggests that contamination through a defect in the can is very limited.

Streptococci often are involved in coagulation of the milk. Savage and Hunwicke [19] isolated various species belonging to the *Escherichia-Aerobacter* group as well as coccus forms and a yeast. Nichols [3] found that spoilage due to non-spore-forming bacteria consisted chiefly of gas formation and/or curdling. Various bacteria were isolated from cans showing the defects, but strains of *Escherichia* were the most common cause of gassy spoilage.

A can of evaporated milk which showed a fishy flavor was investigated by Hammer[23] and an organism capable of producing fishiness in milk, cream, and evaporated milk was isolated. In inoculated milk there was coagulation and rapid digestion in addition to development of a fishy flavor. The organism is now designated *Proteus ichthyosmius.*

Hammer[24] obtained a *Streptococcus,* which he designated *Streptococcus distendens,* from a can of evaporated milk showing slight gas formation. In inoculation experiments it never produced sufficient gas to burst a can; although it grew well in sterile skim milk, gas formation was not noted unless the milk was sealed in some way, for example, with a mixture of Vaseline * and paraffin. *S. distendens* was isolated from evaporated milk in which other non-spore-forming organisms also were growing so it probably gained entrance after the heating.

It should be noted that in a can of evaporated milk slight gas development, which might go unnoticed in a tube of milk, makes itself evident because all the gas produced is held. Inoculation into a can of milk thus affords a convenient method of detecting slight gas formation with organisms that will grow in this material.

During World War II spoilage of evaporated milk due to defects in the containers was relatively extensive. Because of the shortage of tin, a thin tin coat was used and, accordingly, there was a greater than normal opportunity for defects in it.

Control of spoilage. Control of spoilage in evaporated milk that is definitely due to an organism surviving the heat treatment, and not to some such irregularity as defective containers, requires answers to a series of questions, including the following: (1) Has there been an unintentional decrease in the sterilization exposure due to variations in thermometers or some other cause? If so, a correction is indicated. (2) Have pipe lines or other equipment been given less complete cleaning and sterilization, or has concentrated or unconcentrated milk been held under conditions favoring growth of heat-resistant organisms, thus permitting a build up of types difficult to kill? The high heat resistance of certain spoilage organisms grown at about their optimum temperature should be regularly recognized in operating a milk plant putting out a product intended to be sterile. (3) Are the opened cans of spoiled milk being so handled that all danger of contamination of equipment, milk, cans, etc., with organisms in the product is avoided? The organisms can be carried on the hands and clothing of employees, by flies and insects generally, and in various other ways.

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* Vaseline is a protected trade name for the product of the Chesebrough Manufacturing Company.

As soon as an outbreak of spoilage develops, all possible precautions should be instituted, without waiting for a detailed analysis of the problem. Eventually, a thorough review of the processing during the period of spoilage may indicate the probable cause of the irregularity.

STERILIZED MILK AND CREAM

Unconcentrated milk and also cream are sterilized in cans. The products have been produced more extensively in Europe than in the United States. With essentially the same sterilization procedure being used as for evaporated milk, the same types of organisms occasionally cause spoilage and the same bacteriological problems are involved.

Avoset. Avoset is a stabilized cream that is packaged after sterilization. Fresh cream,[25] with sodium alginate added, is sterilized in a continuous flow by direct application of high-pressure steam. In the first units of the equipment, the cream is preheated, under pressure, in two kettles. It then flows to the sterilizers and is processed at 126.7 to 137.8 C (260 to 280 F). The sterilization period may vary but seldom is over 4 min. The sterilized cream then flows to the homogenizer, where the pressure is kept below 2,000 lb in the first stage and below 500 lb in the second. Temperature of homogenization is not critical but should be above 65.6 C (150 F), whereas pressure must be carefully gauged to avoid too much viscosity in the product. From the homogenizer the cream flows into cooling coils and then into sterile holding tanks that feed the bottle filler. In the bottling room radiations from ultra-violet lamps guard against product contamination. Operators wear sterilized uniforms, gloves, and masks. Bottle caps are first loaded into filling magazines, then placed with bottles in retorts and steam sterilized at 135 C (275 F). The bottles are placed on an endless conveyor protected by a bank of germicidal lamps which takes them through the filling and capping machines that perform sterile operations. The entire room is regularly scrubbed and steamed. Air entering the bottling room is specially cleaned and conditioned. After capping, the bottles travel over an air-cooled conveyer which removes most of the heat from them. Laboratory tests establish the bacteriological and physical qualities of the product.

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Chapter 11

Bacteriology of Sweetened Condensed Milk

For use in areas where fresh milk is not readily available, for storage at ordinary temperatures, and for preservation during periods of surplus, whole or skim milk is converted to a sweetened condensed product and put in cans or barrels. The keeping qualities of this product depend primarily on the concentration of added sucrose or, occasionally, of other sugar and to some extent on the concentration of milk solids; destruction of organisms during the condensing also may be a factor. The plasmolyzing action of the materials in solution effectively prevents development of most organisms. This is shown by the spoilage of diluted sweetened condensed milk under conditions with which the undiluted product shows no change. Sweetened condensed milk keeps for considerable periods when it is allowed to stand open at temperatures satisfactory for growth of many micro-organisms, and this is one of its important advantages in areas with limited refrigeration facilities.

Microbiological examination. If a can of milk is to be cultured, it must be opened with care to avoid contamination; the procedure used with evaporated milk is satisfactory. Collection of samples from barrels also requires care if contamination is to be avoided. Ordinarily, cultures should be made in bouillon or on agar containing large amounts of sucrose (30 to 40%) to simulate the high osmotic pressure in sweetened condensed milk; because organisms normally are present, plate counts on agar containing considerable sucrose frequently are desirable.

In investigations on microbiological spoilage in sweetened condensed milk, microscopic examination of smears made from the diluted product (for example, 1 part to 5 parts distilled water) may reveal useful information, such as the predominant organisms, actively budding yeast cells, etc. With certain stains and careful observation, some of the dead bacterial cells commonly can be seen. Leucocytes are easily seen in stained preparations.

Since various organisms can be present in sweetened condensed milk in relatively large numbers without affecting it appreciably, well-controlled tests are necessary in establishing a particular organism as the cause of a defect.

Importance of sugar concentration. Practical experience has shown repeatedly that with low sugar concentrations spoilage is common. Rice and Downs [1] investigated the importance of sugar concentration in the keeping qualities of sweetened condensed milk. The percentage of sugar in solution in the water was calculated from the percentages of sugar and water, and was designated the sugar ratio; for example, a sample with a sucrose content of 46.6 per cent and a moisture content of 27.4 has a sugar ratio of 63.0. In order definitely to prevent growth of an organism causing thickening in sweetened condensed milk it was necessary that the sugar ratio be at least 64.5. As the ratio fell below this the organism became more and more active until at 63.0 a strong odor developed and solidification began.

The organism isolated by Hammer [2] from gassy sweetened condensed milk grew in saturated sucrose bouillon but failed to produce gas in one of the brands of sweetened condensed milk into which it was inoculated. This suggests that the milk solids may be a factor in restraining growth as well as the sucrose, especially since the brand in which the organism would not form gas was one high in milk solids.

Some lots of sweetened condensed milk keep with a sugar ratio lower than the one suggested by Rice and Downs. Low holding temperatures favor keeping; the types of organisms present also are a factor and these can be greatly influenced by the care used in the processing.

Normal sweetened condensed milk. Various investigators have noted that sweetened condensed milk lacks sterility and a few have reported the numbers of organisms found, which vary from less than 100 to 100,000, or more, per milliliter. It is probable that the quality of the materials employed and the care used in handling are important factors in determining the numbers. Cocci of various types probably have been the most common organisms reported but a number of others also have been isolated. Weinzirl [3] noted that sweetened condensed milk contained *B. mesentericus* and *B. subtilis.* Downs [4] stated that the bacterial count, determined by the plate method, of sweetened condensed milk shortly after its manufacture is a good indication of the sanitary conditions of the plant producing it.

Coutts [5] considered the growth of bacteria in sweetened condensed milk. He referred to the work of Sandilands, who found only slight increases, and to the work of Heggs, who noted small increases with most brands but an increase from 21,800 to 114,700 per milliliter in 3 days at 22 C (71.6 F) with one brand. Andrewes[6] found that *Staph. aureus* increased greatly and his experiments suggest that condensed milk is almost a differential medium for growth of staphylococci.

It is evident that sweetened condensed milk contains organisms and these may undergo some growth and the product still remain normal. How extensive the growth can be without the product becoming abnormal presumably depends on the types of organisms involved and perhaps other factors. General laboratory experience indicates that micrococci are the organisms most often multiplying in the normal product and that the holding temperature definitely influences the rate of multiplication; apparently, several hundred thousand of the organisms per milliliter sometimes are present without a conspicuous defect being noted. Extensive increases in numbers usually are followed by decreases which may be rather rapid.

Types of spoilage due to organisms. The changes which occur most commonly in sweetened condensed milk as a result of activity of micro-organisms are gas formation, thickening, and development of molds.

Gas formation. Development of gas in sweetened condensed milk often is extensive and results in pronounced bulging of the cans and sometimes in bursting of the seams. In certain instances the fermentation has been very serious because it appeared suddenly and involved practically the entire pack for a number of days. The organisms usually responsible for development of gas in sweetened condensed milk are yeasts that ferment sucrose but not lactose.

Pethybridge[7] isolated two types of yeasts from gassy sweetened condensed milk put up at an Irish condensery. Each type formed gas in sweetened condensed milk and in saturated sucrose bouillon. The predominant yeast was oval; it produced gas from dextrose and sucrose but not from lactose, and did not grow well in the absence of fermentable sugar. Inoculated tubes of sweetened condensed milk showed gas formation in about 19 days at 37 C (98.6 F) or 24 days at 20 C (68 F). The other yeast was spherical; it fermented sucrose but not lactose and grew fairly well on sugar-free media. Gas was produced in sweetened condensed milk in about 20 days at 37 C.

Yeasts were obtained from gassy sweetened condensed milk by Hunziker.[8] In sucrose bouillon they caused violent gas formation.

Hammer[2] named the organism studied by him *Torula lactis-condensi*. It was isolated from a very serious outbreak of blown milk involving about ten lots packed at one plant; 7 to 8 days elapsed between canning of the milk and development of pronounced swelling. The defect largely, but not entirely, involved milk that had passed through a certain filler in which it was more exposed than in the other filler used in the plant. Development of gas was so extensive that the cans were greatly bulged and often ruptured along the seams. When swollen but unbroken cans were punctured, milk and large volumes of gas foamed through the opening for a considerable period. The fermented milk had a yeasty flavor but was not disagreeable, and there was no suggestion of putrefaction. A yeast was evident microscopically and was readily isolated by plating the material on beef extract agar to which 30 per cent sucrose had been added. Inoculation trials with one brand of sweetened condensed milk gave only negative results but with another brand gas formation usually resulted. The organism grew well in bouillon to which 30 per cent sucrose had been added and produced considerable alcohol in the medium; it also grew in bouillon saturated with sucrose. Results were obtained that indicate that yeasts capable of growth in 30 per cent sucrose bouillon can be rather commonly isolated but that most of them cannot produce gas in sweetened condensed milk.

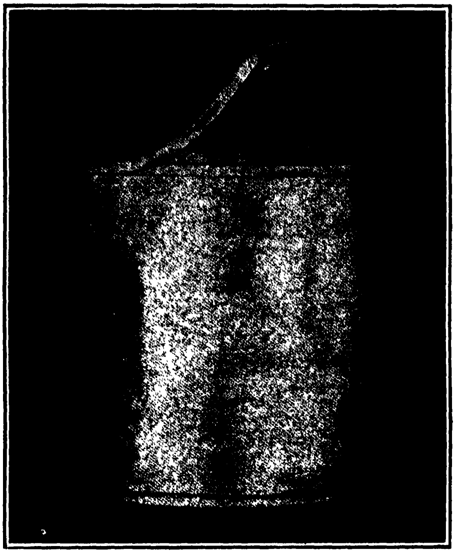

FIG. 24. Can of sweetened condensed milk blown open after artificial inoculation with *Torula lactis-condensi*. Iowa Agr. Exp. Sta. Res. Bul. 54.

Two types of yeasts were isolated from gassy sweetened condensed milk by Knudsen.[9] Both types produced gas in sweetened condensed milk and fermented dextrose, levulose, and sucrose, but not galactose, maltose, or lactose. From Danish sweetened condensed milk only a spherical type was obtained; from Dutch sweetened condensed milk both a spherical and an oval type were isolated. The spherical type developed gas in a sucrose substrate between 7 and 35 C (44.6 and 95 F), showed good surface growth on gelatin with slow liquefaction, and produced alcohol slowly in 30 per cent dextrose bouillon. The oval type developed gas in a sucrose substrate between 14 and 35 C (57.2 and 95 F), showed no surface growth on gelatin, and produced alcohol rapidly in 30 per cent dextrose broth. Knudsen assumed that the yeasts were identical with those found by Pethybridge.[7]

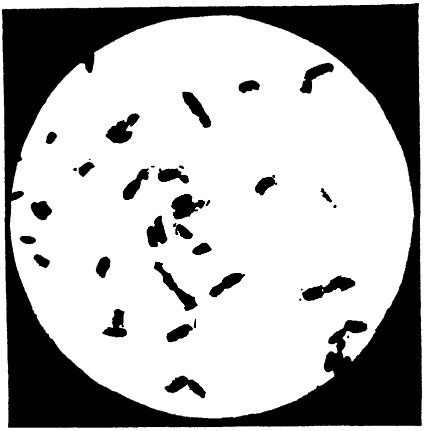

FIG. 25. *Torula lactis-condensi.*

Hiscox[10] obtained two yeasts capable of producing gas in milk containing 70 per cent sucrose and in sweetened condensed milk. One, a spherical type, developed a heavy white growth on beer-wort agar; the other, an oval type, developed poorly on this medium. Both types fermented dextrose, levulose, and sucrose but not maltose or lactose, and action on galactose was questionable. Milk, with up to 70 per cent sucrose added, showed vigorous gas formation followed by coagulation. Inoculated cans of sweetened condensed milk held at 22 C (71.6 F) bulged after 10 to 17 days with the spherical type and after 4 days with the oval type. The two yeasts were compared with the organisms isolated by Knudsen[9] and found to be identical.

Olson and Hammer[11] studied six samples from an outbreak of gassiness in sweetened condensed milk that was marketed in barrels. Development of gas occurred more or less frequently in the milk packed during several summer months. Most of the defective lots did not develop gas until some time after they had left the plant. All the samples yielded an oval yeast and one of them also contained a spherical type. Two samples of gassy milk from another source yielded the oval yeast and one of them also contained a spherical yeast. The oval type was identified as *T. lactis-condensi.* The name *Torula globosa* was suggested for the spherical type which apparently is identical with the spherical yeast isolated from gassy milk by various investigators. The authors concluded from their studies and reports on previous investigations that gas formation in sweetened condensed milk commonly is due to *T. lactis-condensi* or to *T. lactis-condensi* and *T. globosa* growing together, and that when the two species are together *T. lactis-condensi* is the more numerous. They noted, however, that Knudsen [9] found only the spherical type in a sample of Danish blown milk. In some instances Olson and Hammer failed to obtain gas formation when *T. lactis-condensi* or *T. globosa* was inoculated into a can of normal sweetened condensed milk; this may have been due to the cultures losing their ability to grow in high concentrations of sugar and other materials since a culture of *T. lactis-condensi* that had been subcultured more or less regularly for about 17 yr had lost its ability to produce gas in a can of normal milk. In a viscous material like sweetened condensed milk the difficulty with which the organisms are distributed may be a factor in limiting their growth.

Rogers and Clemmer [12] found an *Escherichia-Aerobacter* organism producing gas in sweetened condensed milk containing 40 to 45 per cent sucrose. The temperature of the milk in the forewarmer was high enough to destroy the organism but inspection of the plant experiencing the defect showed abundant opportunity for infection, particularly by flies, between the pan and the can filler.

The available data show that the organisms generally responsible for gas formation in sweetened condensed milk are not sufficiently resistant to survive the heat employed in the manufacturing process, so that contamination after the condensing must be the cause of the abnormality. Contamination probably most often comes from the plant equipment. In an outbreak studied by Hunziker [8] the crust of sugar on the inside of the chute carrying sugar from the floor above contained practically a pure culture of the yeast present in the spoiled milk.

Thickening. Sweetened condensed milk sometimes undergoes thickening. In the early stages only portions of the milk in a can may be involved but later the milk may be thick throughout and pour only with difficulty. Thickening sometimes is the result of a physical change that is influenced by various factors, such as season of production, high storage temperature, long holding, acidity, salt balance, etc., but also can be due to action of micro-organisms. With continued growth of the organisms causing thickening, there usually is development of an abnormal flavor.

Rice and Downs[1] studied bacterial thickening of sweetened condensed milk and found it due to a coccus that is capable of inverting and destroying sucrose and producing considerable acid. It grows in fairly high sucrose concentrations but can be checked if the concentration is sufficiently high; at temperatures above normal room temperature it is particularly active. The organism was isolated from factory equipment which suggests that contamination of the milk may come from this source.

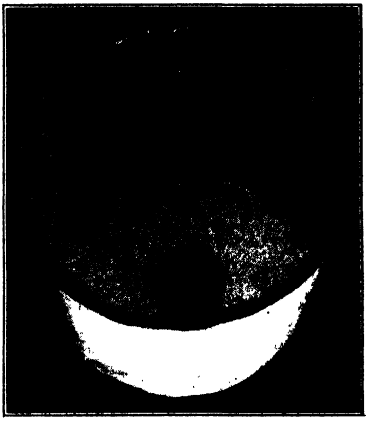

FIG. 26. Buttons on sweetened condensed milk. Courtesy A. M. Besemer.

Downs[4] reported that the organisms thickening sweetened condensed milk belong to one general type of *Micrococcus* of which two distinct varieties were noted. Many organisms of common occurrence closely resembled these and could only be distinguished from them by their effect on sweetened condensed milk. Downs considered that the thickening is due to production of a rennin-like enzyme by the responsible organisms; the nonimportance of acid is shown by the failure of certain organisms producing a high acidity in the milk to thicken it, and also by the sweet curdling of litmus milk in which the organisms causing thickening have grown. Exposure to 64 C (147.2 F) for 10 min killed the organisms in broth.

Development of molds. Molds may grow in cans of sweetened condensed milk, either on the surface of the milk or on milk adhering to the side or top of the can. Some molds form colonies without any significant effect on the milk; these may be conspicuous or they may be very inconspicuous and consist of only a small mycelial growth and a few spore heads. Other molds coagulate the milk in the areas of the colonies to form masses that are referred to as buttons. They may lie free in the milk so that they can be easily lifted or they may be rather firmly attached to the side or top of the can. Apparently, a button may continue to increase in size long after the mold ceases growth, due to diffusion of the mold protease. Milk near mold growth often has a brownish color and objectionable odor and flavor. Buttons sometimes are conspicuously brown.

Molds grow rather slowly in cans of sweetened condensed milk, even at rather favorable temperatures, presumably because of the limited oxygen supply. Growth is most conspicuous when the cans are left in one position for an extended period. If the cans are inverted occasionally, growth tends to be less extensive and some of the buttons may be imbedded in the mass of milk so that they are not easily seen. Presumably, many buttons in sweetened condensed milk are missed by the consumers because of the manner of opening the can or of using the contents.

In underfilled cans mold growth may be very conspicuous due to the increased air supply. It appears that relatively rapid and extensive growth of coccus forms in sweetened condensed milk tends to limit, or entirely prevent, growth of molds, presumably by quickly using the oxygen in a can.

Rogers *et al.*[13] found that buttons are due to growth of *Aspergillus repens* and possibly other molds and that using of the air in a can prevents continued development of the mold. They suggested that development of buttons can be prevented by keeping out the molds through careful methods or by making the conditions unsatisfactory for their growth, either by use of a low holding temperature or by excluding oxygen. The investigators noted that buttons did not develop in milk held at 20 C (68 F) and that sealing the cans under a vacuum of 20 in., or more, prevented their formation.

Knudsen[9] reported that in Europe buttons in sweetened condensed milk often could be traced to *Aspergillus glaucus;* another mold, *Catenularia fuliginea,* was found which forms somewhat similar buttons.

About 40 cans of sweetened condensed milk from a factory that had encountered buttons were examined by Nichols[14] who found 77 per cent of the cans, sampled at random from 15 days' manufacture, showed buttons. Three species of molds were isolated, one belonging to the genus *Actinomyces* and two to the genus *Penicillium.*

Other defects. A fruity flavor sometimes is encountered in sweetened condensed milk. It varies in degree and when conspicuous may be accompanied by lumps of thickened milk. Presumably, the condition is due to coccus forms that succeed in growing rather extensively in the product.

Nichols[14] studied a can of sweetened condensed milk that had a putrid flavor, suggestive of bacterial proteolysis, and was considerably thinned; the plate count was 267,000 per milliliter and a positive presumptive coliform test was obtained with 0.1-ml amounts. Nichols[14] also encountered cans that lacked the freshness of flavor which is usually characteristic of sweetened condensed milk.

Control of microbiological defects. Since certain bacteria can cause defects in sweetened condensed milk, the total numbers should be kept to a minimum by adequate precautions with the milk supply, equipment, etc.; with very few bacteria present, the chances of including spoilage types are small. The very serious losses that have been caused by sucrose-fermenting yeasts suggest that these organisms should be carefully avoided. Although certain sucrose-fermenting yeasts cannot grow in sweetened condensed milk and other yeasts apparently are of no importance in the product, keeping the total numbers of yeasts to a minimum reduces the danger of including harmful types.

Control of mold growth in sweetened condensed milk is an important problem. It involves destruction of the molds in the raw materials by the heat treatment, prevention of contamination during the processing, and canning or barreling of the product without contamination. In some plants contamination during processing apparently has been the cause of the difficulty, whereas in other plants exposure during canning to air containing mold spores has been responsible. Treatment of the air entering the canning room to reduce greatly the numbers of organisms in it is an important consideration. Use of cans that are practically full when sealed limits the air supply of any molds that may be present and appears to be a useful procedure.

Enzymatic and chemical changes. Both enzymatic and chemical action may be involved in the deterioration of sweetened condensed milk. Results of Rice and Markley [15] indicate that rancidity is due to the action of milk lipase and Rice [16] reported data supporting this conclusion. The enzyme hydrolyzes the milk fat and thus forms fatty acids, to some of which the rancid flavor is due. Rice found that the addition of only 0.75 per cent unheated milk to a batch of sweetened condensed milk may cause rancidity; this was equivalent to 0.3 per cent of the amount of whole milk from which the lot was prepared. Addition of pancreatic lipase produced the same result but the condition was not produced by the organisms used. Heating whole milk to 60 C (140 F) for 15 min or 65.6 C (150 F) for an instant destroyed the lipolytic power, but when sugar was present or when condensed milk was heated a higher exposure was required. Rice considers that leaky intake valves and carelessness in heating the milk in the hot wells are factors that may result in the presence of active lipase in the finished milk.

Tallowiness in sweetened condensed milk was investigated by Rice,[17] who pointed out that it is due to action of the oxygen of the air on the butter fat, the reaction ordinarily being catalyzed by small amounts of copper. Biological agencies were excluded as a cause of tallowiness because (1) the condition apparently developed at 0 C (32 F) as rapidly as at room temperature, (2) neither heat sterilization nor strong preservatives prevented it, and (3) bacterial counts on tallowy samples were low. Tin did not catalyze the reaction, whereas iron did to a much less degree than copper.

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Chapter 12

Bacteriology of Milk Powder

In the preparation of milk powder, or dry milk, the reduction in bulk for purposes of storage or transportation is carried to the extreme since there is only a very small percentage of moisture in the finished product. Milk powder antedates both evaporated milk and sweetened condensed milk, its history going back to medieval times when it was prepared by very simple procedures. At present, milk is dried on rolls and also in elaborate spray-drying equipment of various types. Both whole milk and skim milk are dried.

The bacteriology of milk powder is relatively simple because (1) the usual manufacturing procedures result in destruction of many of the organisms in the original milk or added from various sources, and (2) the low moisture content prevents growth of the organisms that survive the processing and is an important factor in causing a decrease in the numbers of those in the fresh powder.

Determination of number of bacteria. Both the plate and microscopic[1] methods have been adapted to the examination of reconstituted milk powder. Ordinarily, a relatively large sample, such as 10 or 11 gm, is more representative than a 1-gm sample because of the variations from one point to another in a can or barrel; milk powder cannot be mixed uniformly as readily as fluid milk. Results of bacterial counts are calculated to a 1-gm basis or, for certain purposes, to 1 ml of reconstituted milk; the 1-ml calculation makes it easy to compare the counts with counts on market milk.

Plate count. The making of plate counts on milk powder has been studied by a number of investigators,[2,3,4,5,6] largely because of the difficulty in dispersing properly certain lots of powder. Without proper dispersion it sometimes is difficult to distinguish between powder particles and small colonies. Warm dilution blanks, dilution blanks of $N/10$ lithium hydroxide, and beads in the blanks have all been advocated to aid in the dispersion. Dilution blanks of 1 per cent sodium citrate solution and containing beads normally are very satisfactory; with powders that are difficult to disperse, the blanks can be warmed to 40 or 45 C (104 or 113 F).

Thiel and Pont[6] reported that concentrations of lithium and sodium hydroxides which effected complete dispersion of milk powder appreciably reduced plate counts. With 3.5 per cent sodium citrate in tap water, a clear plate was obtained even with a 1 to 100 dilution, and there was no reduction in plate counts in tests on fresh powder. Preliminary warming of blanks to 40 C was necessary to disperse roll powder.

Recently, certain investigators[7] have emphasized the importance of incubating plates at 30 C (86 F), as well as 37 C (98.6 F), for the detection of organisms that fail to develop at 37 C.

Microscopic count. Material for direct counts can be dispersed advantageously in $N/10$ lithium hydroxide or in 1 per cent sodium citrate. With most of the organisms dead as a result of the heat treatment, special stains and good lighting are advisable to make the cells more readily seen.

Supplee and Ashbaugh[8] employed a microscopic method, the milk powder first being mixed with enough water to give the concentration of solids found in natural milk. With milk dried by the Just atmospheric roll process, the method showed from 30 to 78 per cent (avg 45.3%) of the organisms present in the original milk. There was a tendency for the groups of bacteria existing in natural milk to be broken during the drying. Jephcott *et al.*[9] found relatively few bacteria in the microscopic examination of roll powder and concluded that the majority of the organisms actually are destroyed or rendered unstainable in the drying.

North[10] regularly obtained higher microscopic counts on milk powder with aniline oil methylene blue * than with plain methylene blue. With 30 (88.2%) of 34 samples, the counts were at least twice as high, with 12 (35.3%) they were at least 5 times as high, and with 3 (8.8%) they were at least 10 times as high. When smears stained with plain methylene blue were decolorized and restained with aniline oil methylene blue, the counts on 26 (76.5%) were more than doubled, on 11 (32.4%) they were at least 5 times as high, and on 3 (8.8%) they were 10 times as high. With raw milk there was no significant difference between the counts obtained with the two stains. North noted that the aniline oil stain has the definite advantage of producing a pale blue background against which the organisms are deeply stained, there is little or no chance of overstaining, and the smears require no special handling but may be transferred from xylene to alcohol and then to the stain with no intermediate steps.

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* Aniline oil 3 ml, ethyl alcohol (95%) 10 ml, 1.5 ml concentrated hydrochloric acid added slowly to the aniline oil and ethyl alcohol, 30 ml saturated alcohol solution of methylene blue, and water to equal 100 ml.

The results of various laboratories indicate that special stains are needed in microscopic counts on milk powder, rather than some of the stains extensively used in counts on raw milk, and that careful observations are necessary. Even with the best technic, not all the organisms in milk powder, as a result of their presence in the original milk, in concentrates made from it, in equipment, etc., can be seen in preparations from it; presumably, the heat treatments are responsible. The percentage of the total organisms that can be seen apparently varies with the types of organisms, heat treatment, staining procedure, etc.

Tests for special organisms. Milk powder sometimes is examined for thermophiles by incubating plates at 55 C (131 F). This procedure is intended to detect organisms that may have grown during the holding of milk or concentrate at relatively high temperatures for excessive periods. Examinations also are made for coliform organisms with the special media employed for this group,[1] the idea being that coliform organisms indicate inadequate heat treatment or recontamination.

Sorensen [11] obtained the thermophilic spore count on skim-milk powder by autoclaving the 1 to 10 dilution of the powder at 5-lb steam pressure for 10 min and plating, with incubation of the plates at 55 C. Use of *N*/60 sodium or lithium hydroxide in the dilution blanks eliminated undissolved flakes or specks in plates and had no detrimental effect on the count. The usual thermophilic count was obtained by plating before heat treatment of the dilution blank.

Numbers of organisms in milk powder. The numbers of organisms in milk powder vary widely. In general, roll-dried powder has lower plate counts than spray-dried powder, which is in agreement with the exposures to heat in the two processes.

Plate counts. Downs [12] investigated the bacterial content of milk powder and found that 10 samples of single roll milk (milk previously condensed) contained 16,900 to 626,000 per gram, with an average of 49,500; 10 samples of spray milk (milk previously pasteurized and condensed) contained 15,600 to 595,000 per gram, with an average of 178,000; and 9 samples of spray milk (milk not previously condensed) contained 1,500,000 to 3,000,000 per gram, with an average of 2,269,000.

Supplee and Ashbaugh [13] examined fresh milk powder made in different factories by the Just double roll process, using 10 to 34 samples per plant. The minimum counts for the various plants ranged from 350 to 2,100 per gram, the maximum counts from 1,400 to 170,000, and the average counts from 790 to 27,300. These investigators also studied the comparative bacterial counts of liquid milk and the milk powder obtained from it. In 10 comparisons the liquid milk contained 1,520,000 to 345,000,000 bacteria per milliliter while the milk powder contained 200 to 1,350 per gram. In studies on the contamination of 7 samples of milk powder they noted counts from 50 to 1,100 per gram (avg 563) on the milk from the cylinder, from 300 to 3,000 (avg 1,614) after sifting, and from 500 to 5,200 (avg 3,271) after packing. The results indicate that, on a percentage basis, contamination of milk powder during its preparation for market is considerable.

Macy[14] studied 31 samples of milk powder prepared by the spray process and obtained counts from 4,400 to 5,500,000 per gram, with the majority of the counts more than 50,000, whereas 13 samples of roll powder yielded counts from 40 to 7,900 per gram, with the majority below 500. According to Supplee and Bixby,[15] milk dried by the Just roll process rarely has a bacterial count of more than 1,000 per gram; a slight increase in count may take place from the time the milk powder leaves the cylinders until it is packed. Eight samples of spray process powder, purchased in the open markets of four states, showed total counts from 24,000 to 175,000 per gram and an average of 75,800.

Nichols[16] found counts from 1,400 to 149,000,000 per gram with 405 samples of spray-dried powder from eight factories. With 671 samples of spray-dried skim milk, whole milk, and whey powders, Crossley and Johnson[5] obtained counts from 200 to 19,500,000 per gram. Higginbottom[17] studied some 400 samples of roll-dried skim milk, whole milk, buttermilk, and whey and spray-dried whey and for comparison included results recorded by Nichols for spray-dried milk. The spray-dried products had strikingly higher plate counts than the roll-dried products and also gave three times as many positive presumptive coliform tests. Marked differences were noted in counts on samples from different plants, some plants giving consistently low counts and others giving higher and more variable counts.

During recent years, plate counts on milk powder have decreased, presumably as a result of the heat treatments used in processing and the control of growth and contamination during handling. Bacterial standards for the product have stimulated studies on sources of the organisms in milk powder and methods of control. With adequate procedures, milk powder having low plate counts, in the hundreds or low thousands per gram, regularly can be produced by either the roll or the spray process, even when the raw milk contains many bacteria. In general, the roll process gives the lower counts. If irregularities in the processing occur, high plate counts may result; they occur more frequently with spray drying.

Microscopic counts. On 33 samples of milk powder, North[10] reported microscopic counts that ranged from 1,890,000 to 2,160,000,000 per gram with the aniline oil methylene blue stain; 2 counts were over 1 billion, 16 additional were over 100 million, 12 additional were over 10 million, and only 3 were under 10 million.

General results secured in various laboratories indicate that high microscopic counts are obtained on some lots of milk powder, even when the plate counts are very low. Apparently, the percentage of the bacterial cells originally present that can be stained after drying varies rather widely, even with the same processing procedure, but microscopic counts on milk powder show general differences in bacteriological quality of the product going to the drier. It should be recognized that the milk as received from the farms is only one factor in determining this quality; the milk, or the concentrate from it, may be so held in the plant that bacteria multiply extensively, serious contamination may occur, etc.

Types of organisms in milk powder. The types of organisms in milk powder have been studied extensively. Nichols[16] found that 80 per cent of 1,304 cultures picked from plates incubated at 37 C (98.6 F) and 74 per cent from plates held at 55 C (131 F) produced acid and coagulation in litmus milk. Of the cultures from plates incubated at 37 C which produced acid coagulation in milk, more than 90 per cent were heat-resistant streptococci; 67 per cent were strains of the "viridans" division (57 per cent being *S. thermophilus*) and 33 per cent were strains of the "enterococcus" division (26.5 per cent being *S. durans*). The milk-curdling organisms from plates incubated at 55 C were *B. calidolactis.*

With 671 samples of spray-dried skim milk, whole milk, and whey powders, Crossley and Johnson[5] noted that the flora on agar at 37 C was of a specialized type and comprised comparatively few species. Thermoduric streptococci predominated, *S. durans* and *S. thermophilus* being the most common species. In addition, five species of micrococci, probably non-thermoduric, also occurred frequently. Organisms of the genus *Bacillus* and three *Achromobacter* species were found regularly, but their numbers were not of practical importance. Spore-forming anaerobes were found in 14 per cent of the samples, probably in small numbers, and were most frequent in winter. Coliform organisms were rare in 1-ml portions of reconstituted milk but with 20-ml portions were found in 25 per cent of the samples. Examination of

198 coliform strains from powders and 164 strains from raw milk showed that *E. coli* types occurred less frequently in the powders, whereas *Aerobacter* strains, especially *A. cloacae,* were most frequent; 48.5 per cent of the strains from powder were heat resistant compared to 2.2 per cent of the strains from raw milk. The coliform flora of powder was due partly to plant contamination by heat-resistant strains, although some non-heat-resistant strains survived spray drying.

Higginbottom[17] reported that cultures from plates made from roll-dried products and incubated at 37 C consisted chiefly of micrococci, sarcinae, and spore-forming bacilli, with a small percentage of non-spore-forming rods, chiefly *Achromobacter* types; a species of *Actinomyces* and streptococci also were found. The flora was markedly heat resistant. Cultures from plates made from spray-dried whey and incubated at 37 C consisted almost entirely of *S. thermophilus* and *S. fecalis.* On plates incubated at 55 C all the products showed mainly spore-forming rods of two principal types, with diverse biochemical properties; strains of *B. subtilis* and a thermoduric *Actinomyces* species also were present in small numbers.

In the studies of Mattick *et al.*[7] the flora of spray-dried powder included streptococci, micrococci, and spore-forming rods. With improvements in plant cleaning, the relative numbers of streptococci declined. With a preheating temperature of 73.9 C (165 F) and a clean plant, micrococci predominated over the streptococci, whereas with a preheating temperature of 87.8 C (190 F) streptococci predominated and spore formers were relatively numerous.

Thiel and Pont[6] reported that spray-dried powder showed almost entirely streptococci, with only a few micrococci and sarcinae, whereas with roll-dried powder the organisms were predominantly micrococci, sarcinae and spore-bearing types, with very few streptococci. Many fresh powders, especially spray-dried skim milk powder, gave coliform tests with 2-gm samples; 0.1-gm samples were almost uniformly negative.

Nichols[16] found the numbers of yeasts and molds in spray-dried powder were practically negligible. In the samples studied by Crossley and Johnson,[5] a few mold species occurred sporadically in extremely small numbers and yeasts were rare. Higginbottom[17] found an occasional yeast and noted that molds did not prove a reliable indication of contamination subsequent to drying. Thiel and Pont[6] observed yeasts in only two samples. Molds ranged from 10 to 60 per gram in fresh powder; they tended to increase, generally to between 50 and 250 per gram, in powders stored at 15 C (59 F) but there was no tendency to increase at 30 or 37 C (86 or 98.6 F).

Sources of organisms. There is a great destruction of organisms during preparation of milk powder, especially with high heat treatments of the original milk or concentrate, but some of the living organisms in the finished product apparently are thermoduric types that were in the original milk. Others may be the result of contamination from equipment, air, handling of the product, etc., and of growth in the milk or concentrate in the plant. Holding concentrated milk at temperatures favorable for growth apparently often has been responsible for excessive plate counts on milk powder. Such holding is especially serious if it favors growth of species that are relatively resistant to heat.

Changes in numbers of organisms. Because the low moisture content makes conditions so unfavorable, a decrease in the bacterial content of milk powder during holding would be expected. Supplee and Ashbaugh [13] concluded that the bacteria in milk powder die off rapidly during storage and that, in normal milk made by the Just process, the numbers reach an approximate constancy after 2 to 4 mo. Milk powder to which organisms were added showed a similar decrease but a longer period was required to reach an approximately constant number. Macy [14] reported a remarkable decrease in counts with both spray and roll powders after storage for 1 yr. The decrease continued and the maximum reduction in counts (99.9%) for the spray process milk occurred after 5 yr, whereas the maximum (97.9%) for the roll powder was reached after 3 yr.

Nichols [16] noted that during prolonged storage the viable bacteria in milk powder died. The mean percentage reduction in counts after 12 mo at room temperature was 95 per cent. The bacteria tended to die more quickly as storage temperatures increased and the numbers decreased faster in an atmosphere of air than in one of nitrogen. A variable decline in numbers of bacteria in powder during storage, with some species dying more rapidly than others, was reported by Crossley and Johnson.[5] Spore-forming bacteria survived longest and coliform organisms disappeared rapidly. Higginbottom [18] found with spray-dried and roll-dried milk and whey that there was a general tendency for the plate counts to fall during storage but that many samples showed little change in count after 6 mo, or longer. Also, there was a greater tendency for a decrease in count with roll-dried than with spray-dried products and the decrease was more definite with whey than with milk. Thiel and Pont [6] noted a decrease in total count on all lots of powder after storage, the decrease being more pronounced at the higher temperatures; the thermophilic organisms did not show the same tendency, except when large numbers were present.

Practical experience in various control laboratories indicates that the decrease in the plate count on milk powder during storage often is pronounced, particularly with a high initial count and relatively high holding temperatures. Packing in an inert gas tends to delay the decrease.

Significance of bacterial counts. Low plate counts on milk powder normally indicate thorough heat treatment during the processing and adequate protection against contamination. They give no indication of the bacteriological quality of the original milk because of the great destruction of organisms with normal processing.

Findlay *et al.*[19] found that, with a raw milk supply of poor bacteriological quality, preheating temperatures of 93.3 and 87.8 C (200 and 190 F) greatly reduced the bacterial count in the preheated and condensed milk; reduction in count was less marked with 82.2 C (180 F) and very considerably less with 76.7 and 71.1 C (170 and 160 F). Bacterial counts on spray-dried powders were low with preheating temperatures of 93.3 and 87.8 C; with 82.2 C they were only slightly higher, but with 76.7 and 71.1 C they were relatively high.

Nichols [16] found wide variations in the mean plate counts of powders from different plants and they seemed to be associated with the technic of manufacture. There was a seasonal variation in quality only in the plants in which the general bacterial standard of the powder was poor. Mattick *et al.*[7] reported that the factors that determine the plate count on milk powder appeared to be, in order of importance: (1) plant cleanliness and sterility, (2) preheating temperatures, and (3) bacteriological quality of the raw milk, which in turn affected the hygienic condition of the plant. According to Crossley and Johnson,[5] plate counts on milk powder and incidence of coliform organisms showed a variable increase during a day's run, which became more pronounced after 10 hr of operation; they considered stoppages for plant-cleaning desirable every 7 to 9 hr.

General experience indicates that over rather extended periods plate counts may be relatively high on the products of certain plants and that corrective measures in the operation of the plants reduce the counts materially, without any change in the quality of the original milk. Plate counts on a series of samples taken during a run of powder can be used to detect excessive numbers of organisms from any source, such as the original milk, growth during holding of the milk or the concentrate from it, excessive contamination from equipment, etc. Microscopic counts on the finished powder can be used to determine whether there has been a large increase in the numbers of bacteria followed by their destruction during the heat treatment.

Effect of drying on various organisms. Effect of the drying process on various organisms has been studied by Hunwicke and Jephcott,[20] using an experimental drying plant identical with the factory machines except that the length of the rolls was reduced. They found non-spore-forming bacteria were completely destroyed while bacterial spores were not destroyed in all cases, the number of survivors apparently being dependent on the extent to which sporulation had occurred. Milk powder prepared from milk highly infected with *M. tuberculosis* was incapable of causing lesions in guinea pigs.

In connection with the destruction of organisms during the drying of milk, various attempts to isolate pathogens from milk powder are of interest, although no definite information is available with reference to the milk used for drying. Shrader *et al.*[21] found that the milk powder supply of Baltimore, as represented by 100 samples from widely scattered sources, was free of *M. tuberculosis.* Many of the samples of milk had very high bacterial counts. In a study of dry milk preparations for infant feeding by Giblin and Pourtales [22] no hemolytic streptococci of the pathogenic type were found in the nearly 200 samples examined, and *M. tuberculosis* was not found in more than 200 samples. Average bacterial counts on the various brands ranged from 1,000 to 13,000 per gram. Fuller and France [23] examined several samples of milk powder for *M. tuberculosis* to determine their safety for feeding calves. The spray-process samples were dispersed in water and injected into guinea pigs; the roll-process samples were fed to guinea pigs. None of the animals became tuberculous.

Crossley and Johnson [5] noted that spray drying destroyed many bacteria but could not in itself be relied on to destroy pathogens, since some non-thermoduric species survived.

In considering the effect of drying on organisms, it is necessary to distinguish between actual drying and exposure to heat preparatory to the drying. With spray drying the rapid loss of moisture during the actual desiccation apparently keeps the temperatures low enough to permit survival of some of the organisms that are relatively susceptible to destruction by heat. The percentage of the cells that survive is small and, presumably, they are protected in some way that does not apply to comparable cells which are destroyed, although there is a possibility they have a slightly greater heat resistance. Adequate heat treatment before spray drying favors low plate counts on the finished powder.

Relationship of organisms to quality and keeping quality. Significant flavor deterioration in the original milk, such as development of acid, affects the flavor of milk powder made from it but there is no close correlation between the flavor of the original milk and of the milk reconstituted from the powder. It appears that, commonly, the processing has a greater effect on the flavor of the finished powder than the flavor of the original milk.

Higginbottom[17] found no relationship between the keeping quality and the plate count of reconstituted milk powder. Lea *et al.*[24] reported that improvement in the bacteriological quality of the milk supply and more careful control during its passage through the plant produced some improvement in the keeping properties of powders prepared from milk preheated at a lower temperature but had relatively little effect on the keeping properties of the powder when a higher preheating temperature was used. Also, removal of slime by means of a clarifier did not significantly increase the storage life of the powders. Thiel and Pont[6] observed no consistent difference in either the initial flavor or keeping properties of milk powders that could be correlated with the bacteriological condition, but noted that the possibility of biological factors in the deterioration of milk powders cannot be excluded.

Keeping qualities of reconstituted milk. The wide variations in the numbers and types of organisms in milk powder, and also in the water used for reconstitution, suggest differences in the keeping qualities of the reconstituted products; in some cases the organisms in the water probably have a more marked effect on keeping qualities than those in the powder. Bacteria added to the product from the utensils and equipment used for the reconstitution also may be a factor in the spoilage.

Milk properly reconstituted from bacteriologically satisfactory milk powder and water keeps very well at temperatures commonly used for holding milk. When bacterial changes occur in the milk they frequently result in very objectionable odors. At relatively high holding temperatures, such as 21.1 to 26.7 C (70 to 80 F), objectionable odors may develop rather quickly. With the normal absence of the common lactic acid organisms in the product, growth of organisms producing undesirable changes may be very rapid.

Nichols[16] found that over 90 per cent of the samples of reconstituted milk held at 15.6 C (60 F) remained sweet from 2.5 to 3.5 days. Tests made on the samples after 24 hr indicated the flora of the reconstituted milk did not increase as rapidly as that of raw milk. At 37 C (98.6 F) the predominating flora of the reconstituted milk rendered it acid and clotted it.

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Chapter 13

Bacteriology of Ice Cream

Bacterial counts are employed in controlling methods of producing and handling ice cream * in the same way they are used in supervising milk supplies. Although most of the bacteria in this product, as in milk, are harmless, the general conditions which permit the presence of large numbers of organisms are those most likely to result in inclusion of harmful types or undesirable products of growth. Bacterial counts, in combination with suitable inspection of the plant and the methods of production, make possible the adequate sanitary control of ice cream. The epidemics that have been traced to the product emphasize the necessity of sanitary methods in producing it.

The bacteriological principles involved in the manufacture of ice cream are essentially the same as those involved in the handling of market milk, since bacteria are undesirable in both products and should be kept out as completely as possible. Those that gain entrance should be destroyed or prevented from growing. However, the variety of raw materials used in ice cream and the conditions of manufacture and holding present problems not encountered in the market-milk industry.

Determination of numbers of bacteria. Both the plate method and the microscopic method are employed in determining the numbers of bacteria in ice cream, the plate method being used the more extensively. The detailed procedures have been adapted from those devised for the examination of milk and have been standardized.[1] Sampling methods for ice cream and procedures for examination of ingredients of ice cream also have been developed.

Plate method. With the plate method, the portion of ice cream examined has been either weighed or measured. Fay[2] compared the gravimetric and volumetric methods, using 10-gm, 10-ml, and 1-ml portions. He concluded that the errors introduced by the various sampling procedures are overshadowed by the error of the plating method and that it makes little difference which of the portions is used. A 10-gm portion was suggested for the occasional sample from which air bubbles cannot be eliminated.

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* The term *ice cream* is used to include not only plain ice cream but the great variety of frozen desserts, confections, etc., that are more or less similar to it.

When ice-cream improvers and powders containing rennin are used, determination of the number of bacteria sometimes is complicated by the ice cream becoming a curdled mass when it is warmed. Such samples require weighing and even then the results are not entirely satisfactory since it is difficult to obtain a representative sample, owing to the expression of whey, and the material cannot be distributed uniformly through a water blank.

Various media have been employed in plating ice cream, the agar for milk analysis more extensively than any other. Fay [3] reported that in making counts on ice cream with nutrient agar large numbers of pin-point colonies were noted in low dilutions (1 to 100), whereas in higher dilutions (1 to 1,000) these were not in proportionate numbers, or were absent. On replating the samples on sucrose agar, the pin-point colonies were present in all dilutions and in proportionate numbers; essentially the same results were obtained by using nutrient agar and adding sterilized ice cream to the higher dilution plates. Fabricius and Hammer [4] noted that when nutrient agar was used in plating ice cream and ice-cream mix occasional samples were encountered with which the different dilutions failed to show a reasonable agreement. Serious discrepancies usually involved the 1 to 1,000 and 1 to 10,000 dilutions. Addition of 1 per cent sucrose to the medium eliminated the discrepancies and also resulted in an increase in size of the colonies. In modifying nutrient agar to favor growth of saccharophilic organisms, 1 per cent dextrose also has been used.

The temperature at which the plates are incubated greatly influences the results obtained on ice cream with the plate method. Ayers and Johnson [5] found that 30 C (86 F) for 5 days gave practically double the count obtained at 37 C (98.6 F) for 48 hr. Ellenberger [6] noted that when the plates were incubated 5 days at 30 C the average count of the samples examined was almost 2.5 times the count obtained with an incubation of 2 days at 37 C; with an incubation of 7 days at 20 C (68 F) the count was more than 3 times as high. Pederson and Yale,[7] using an incubation of 48 hr, obtained higher counts at 32 C (89.6 F) than at 37 C.

In employing plate counts for the sanitary control of ice cream, the maximum counts obtainable are not necessary, for the same reason they are not necessary with routine counts on milk, but it is essential that standard methods be followed in detail so that various counts can be compared.

Microscopic count. Fabian[8] adapted the microscopic count to the examination of ice cream. The procedure followed is essentially the same as that for milk.

A modification of the microscopic count, intended especially for ice cream and other viscous dairy products, was described by Fay.[9] One-tenth ml of the sample is placed on a clean slide of the usual size (1 × 3 in.). Two to four drops of water (sterile or boiled) are added and the mixture spread evenly over the entire slide. After drying, the smear is treated in the usual manner. When the diameter of the microscopic field is adjusted to 0.157 mm the bacterial count can be determined by multiplying the average number of cells per field by 1,000,000. If the product contains relatively few organisms it is convenient to count the cells in a strip, the width of the microscopic field, across the short dimension of the slide. In this case the total number of organisms is multiplied by 5,000 to give the count per milliliter. A factor of 5,000 requires a field diameter of 0.152 mm but the slightly larger field compensates for the lack of definition at the periphery.

Microscopic counts on ice cream can be made on either the individual cell or group basis, a group being considered as an unseparated clump, chain, pair of cells, or an individual cell. Although heavily stained cells sometimes are regarded as living and poorly stained cells as dead, this concept has little justification, and microscopic counts on ice cream should be interpreted with caution. The presence of large numbers of bacteria, whether living or dead, is objectionable; most often it indicates high bacterial counts on one or more ingredients.

Burri technic. Long and Hammer[10] applied the Burri smear culture technic to ice cream. Small portions are picked with a sterile needle under a low power binocular and each is spread over the surface of a dry, standard agar slope. The portions are intended to weigh 0.02 mg and are picked from firm ice cream, the product being surrounded with ice if necessary. The binocular aids in keeping the portions fairly uniform in size and in actually picking each portion rather than scraping it from a relatively large area. Ordinarily, 10 portions are picked from a sample but larger or smaller numbers may be used for more or less detailed results. There is considerable variation in the size of the portions picked. Counts with the Burri technic tend to be higher than plate counts. The original surface of the ice cream can be used, a fresh surface can be exposed with a sterile instrument, or the surface and interior can be compared. The method probably has its greatest value in studying the distribution of bacteria in ice cream, particularly in studying the numbers at the surface of dipped ice cream.

Frost little-plate method. Using standard agar, Bryan *et al.*[11] found with ice cream that incubation of Frost little plates at 37 C (98.6 F) in a moist chamber for a minimum of 8 hr gave counts comparable with standard plate counts. In ice cream containing seeds, nuts, or chocolate, particles may confuse the technician unless the product is diluted 1 to 10 or 1 to 100 before making the little plates.

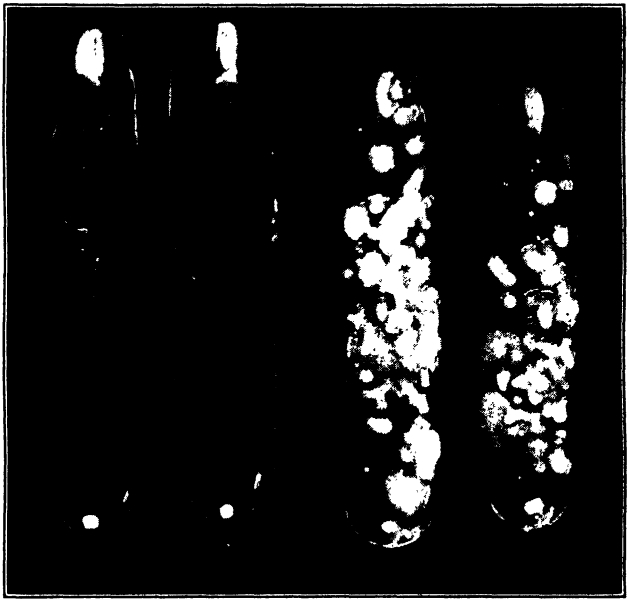

FIG. 27. Agar slopes (Burri technic) from interior and surface of dipped ice cream. Colonies indicate heavy contamination of surface of ice cream.

Coliform counts. Ice cream frequently is examined for coliform organisms, using essentially the methods developed for milk.[1] The organisms often are found in ice cream with very low bacterial counts; the strains may be relatively heat resistant but often they are not, and then probably the organisms represent contamination from equipment, personnel, flies, etc.

Fournelle and Macy [12] found the range of coliform counts, in terms of most probable numbers, on 69 factory-packed samples of ice cream and sherbet was from 0 to 9,180 per milliliter; with 30 scoop samples of ice cream the range was from 0 to 101,000. They concluded the results indicated that ice cream should contain <10 coliform organisms per milliliter. Various coliform species were found.

Reduction tests. Nelson[13] reported that the resazurin test, using as the end point either the period for the color to become completely pink or the color after 3 hr, showed possibilities as a means of segregating samples of ice cream with high plate counts; the 1-hr test showed little promise. Segregation on the basis of the methylene blue test did not seem practical.

Numbers of bacteria in ice cream. Bacterial counts on ice cream vary widely, owing primarily to differences in quality of the materials used and in methods of manufacture and handling. With well-controlled production, plate counts may be as low as a few thousand or even under 1,000 per milliliter; with careless methods they may be in the millions.

During recent years there has been a significant decrease in plate counts on ice cream as a result of the attention given the sanitary quality of the product by manufacturers and control officials. Quality of the ingredients has improved, more efficient pasteurization has been developed, and contamination of the product from various sources has been reduced. The product of a plant occasionally shows a significant increase in count for no apparent reason. However, study of the general operation, with the examination of samples taken at various points, usually indicates the cause of the increase; poorly cleaned equipment, inadequate heat treatment, high-count ingredients added after the pasteurization, etc., may be involved.

Types of bacteria in ice cream. Studies on ice cream have resulted in the isolation of a wide variety of bacterial species, including micrococci, streptococci, aerobic and anaerobic spore-formers, *Escherichia-Aerobacter* types, *Pseudomonas* species, etc. In their early studies on ice cream sold at retail in Washington, Ayers and Johnson[14] picked colonies into litmus milk and found coagulating and non-coagulating acid formers, inert forms, alkali formers, and peptonizers during both summer and winter. Acid-forming organisms made up a considerable percentage of the total flora. In the ice cream investigated by Ellenberger[6] these organisms predominated all through the storage. Fabricius and Hammer[4] inoculated pin-point colonies from 54 samples of ice cream, representing 26 plants, into litmus milk; of the 1,016 cultures obtained, 43.8 per cent were acid coagulators, 47.3 per cent were acid non-coagulators, 2.5 per cent were alkali formers, 6.3 per cent were peptonizers, and 0.1 per cent were inert. Causes of the small size of the colonies varied widely. Some of the organisms were types which regularly yielded only small colonies, others gave only small colonies at 37 C (98.6 F) although they gave larger colonies at lower temperatures, still others gave small colonies on routine plates but yielded large colonies when pure cultures were plated.

From the standpoint of the general types of organisms present, ice cream is comparable to pasteurized milk or cream. If ice cream is melted and held at a temperature favorable for growth, various bacterial species develop and produce conspicuous changes; yeasts and molds also may multiply.

SOURCES OF BACTERIA IN ICE CREAM

The bacteria in ice cream come from two main sources: (1) ingredients used, and (2) contamination incident to manufacture and handling.

BACTERIA IN INGREDIENTS USED

The ingredients that require consideration from the standpoint of the bacteria in ice cream are (1) cream, (2) other milk derivatives, (3) stabilizers, (4) sugar, (5) flavoring materials, and (6) coloring materials.

Cream. The cream employed in ice cream varies widely in bacteriological condition. It may be skimmed at the plant from milk of excellent quality; on the other hand, it may come from milk of poor quality and be shipped, inadequately refrigerated, for long distances. In general, during periods of heavy demand for cream the ice-cream manufacturer must be less critical than during periods of surplus. Pasteurization of the cream and/or ice-cream mix normally destroys a high percentage of the bacteria originally in the cream, including any pathogens that may be present. Both the numbers and percentages of organisms destroyed depend on the species present, exposures used, etc., but regularly are high with satisfactory methods. Pasteurization of the cream and low holding temperatures, such as 0 C (32 F) or slightly lower, make it possible to hold cream for reasonable periods in order to meet any demands that may arise.

Cream reconstituted from frozen cream, butter, butter oil, etc., using fresh milk or milk powder, presents the same general problem as fresh cream. The materials used in the reconstituting contain bacteria in variable numbers, depending on the quality. Adequate heat treatment destroys many of them.

Other milk derivatives. The milk derivatives other than the cream that are employed in the ice-cream mix include especially products intended to raise the content of solids not fat although some of them also influence the fat content. Each one may be the source of many organisms, but some contain large numbers more often than others. With adequate control of the original quality and proper handling, relatively small numbers of bacteria come from these ingredients.

Stabilizers. In general, stabilizers are comparatively unimportant sources of the bacteria in ice cream, particularly since they are used in such small percentages.

At one time the gelatin used in ice cream often contained large numbers of bacteria, counts over 100,000,000 per gram having been reported. Some samples contained small numbers, indicating that production of a low-count product was possible. The types of bacteria present consisted largely of spore formers and micrococci; the low moisture content tended to eliminate organisms not relatively resistant to desiccation.

The heat used in dissolving gelatin destroys large numbers of the contained organisms. This is evident from data reported by Ellenberger[6] on 15 samples of gelatin from manufacturers and dealers. When dissolved at 51.7 to 54.4 C (125 to 130 F) the bacterial counts per gram of dry gelatin varied from 430 to 2,396,000 and averaged 388,162; when dissolved at 71.1 to 76.7 C (160 to 170 F) they varied from 35 to 5,720 and averaged 1,172; when dissolved in boiling water they ranged from 5 to 3,200 and averaged 530. Brannon and Tracy[15] found that heating gelatin at 60 to 71.1 C (140 to 160 F) greatly reduced the number of bacteria present. Fay and Olson[16] pasteurized 22 samples of gelatin at 62.8 C (145 F) for 30 min and noted that destruction of organisms varied from 0 to 99.9 per cent. With one exception all the samples originally containing more than 3,000 bacteria per gram showed a reduction of more than 90 per cent as a result of the heating. The largest number that survived the heating in any sample was 62,000 per gram.

At present, most samples of gelatin contain relatively small numbers of bacteria because of the care used in its production. In general, other types of stabilizers also contain only small numbers and are unimportant as a source of bacteria in ice cream.

If stabilizers are kept dry and properly protected from dust and dirt, the numbers of bacteria present tend to decrease as a result of the death of organisms. If not properly protected, the numbers may tend to increase.

Sugar. In general, sugar contains only small numbers of bacteria and is of little importance as a source of bacteria in ice cream. In the early studies on the bacteriology of ice cream, Ellenberger[6] obtained counts that varied from 20 to 255 per gram; those of Fay and Olson[17] ranged from 20 to 240. Requirements of canners, soft-drink manufacturers, etc., have emphasized the importance of organisms in sugar and resulted in rather adequate microbiological control of the product by manufacturers. Exposure of sugar to dust and dirt in plants undoubtedly results in an increase in the bacterial content.

Flavoring materials. Flavoring materials should be recognized as a source of organisms in ice cream. The early studies of Newman and Reynolds[18] showed the great variations in the numbers of organisms from these materials.

With five samples of vanilla extract Hammer[19] found counts from 200 to 2,300 per milliliter. Prucha[20] examined 13 samples from commercial ice-cream plants; yeast and mold counts ranged from 0 to 20 per milliliter and bacterial counts from 30 to 60,000.

The bacteriological condition of nut meats has been studied extensively because of the conditions under which some of them are prepared. Prucha[20] found that 20 samples of nut meats from commercial plants contained from 0 to 470 yeasts and molds per gram and from 0 to 1 million bacteria. Wenzel and Black[21] noted that treated nut meats kept in closed containers in a cold room had relatively low bacterial counts; those that were raw and kept at room temperature in open containers had high counts. Yeast and mold counts also increased with poor storage conditions. Numbers of anaerobes and thermophiles were negligible. The 33 species of predominating organisms were nonpathogenic; 20 of the species belonged to the genus *Bacillus,* and *B. subtilis* was present in 50 per cent of the samples. *E. coli* was not isolated. Ostrolenk and Hunter[22] reported that nuts in unbroken shells do not contain coliform organisms. They examined 548 samples of nut meats, representing 11 varieties, for *E. coli.* With 200 retail market samples, the organism was isolated from 8 of the 10 varieties of nuts in 0.01-gm portions, the percentages ranging from 4 to 45; with 234 samples from 38 commercial shelling plants, all but one of the varieties showed the organism in from 6 to 68 per cent of the samples; with 114 imported samples all but one of the varieties showed the organism in from 14 to 62 per cent of the samples. Artificially contaminated nut meats stored at room temperature contained viable *E. coli* for approximately 68 days. When large numbers of *E. coli* were present on nut meats, correspondingly large numbers of *A. aerogenes* and intermediates were found.

Tracy[23] concluded that nut meats should be dipped in boiling sugar solution (about 50%), to which 1 per cent salt has been added, for 15 sec and dried in a 250 C (482 F) oven for 2.5 min; prepared nut meats should be stored in glassine bags or tin cans at room temperature.

Clark and Booth [24] reported that the numbers of aerobic organisms on nut meats varied with different samples and with the kinds. Samples from open counters had, in general, much higher counts than those in cellophane bags. Aerobic spore formers were the most common organisms encountered. Seven (3.12%) of 224 samples yielded cultures of *E. coli* and 54 (24%) yielded staphylococci. Artificially contaminated nut meats stored at room temperature contained viable cells of *E. typhosa* for 3 to 4 weeks, *E. coli* for 5 weeks, and *Staph. aureus* for 9 to 10 weeks.

Prucha [20] noted that 13 samples of frozen-pack strawberries contained from 0 to 19,000 yeasts and molds per gram and from 10 to 67,000 bacteria. Nine samples of maraschino cherries had from 0 to 110 yeasts and molds per gram and from 0 to 1,780,000 bacteria. Tracy [23] found 8 samples of fresh strawberries contained from 300 to 839,000 bacteria per gram. He reported that fruits with delicate flavors, such as fresh strawberries and raspberries, can be satisfactorily pasteurized by mixing with sugar and heating at 62.8 C (145 F) for 30 min; if necessary, certain flavor extracts can be heated at 62.8 C for 30 min without serious injury to the flavor.

Coloring materials. The possibility of color solutions adding organisms to ice cream has been considered by various investigators. Fabian [25] found such solutions had counts from 0 to 15 million per milliliter and 35 per cent contained *Escherichia-Aerobacter* organisms. Smallfield [26] pointed out that many of the colors are added after the pasteurization. Powdered colors were found to be relatively free of organisms. Eight samples being used to make up liquid colors in plants produced 0 to 3,300 colonies per gram, whereas 8 samples from supply houses yielded 0 to 6,500. Twenty-six liquid colors prepared in ice-cream plants produced 2,700 to 198,000,000 colonies per milliliter, and age of a preparation was no indication of the number of organisms present. Nine samples of liquid colors obtained from supply houses yielded 0 to 13,750,000 colonies per milliliter on arrival; 6 weeks later some of the counts had increased and others had decreased. Smallfield suggested various methods of controlling the numbers of bacteria added with the colors, such as (1) dissolving the dry color in 45 per cent sucrose solution and boiling 2 min, and (2) adding a mixture of dry color and pulverized sugar directly to the mix in the freezer.

The Illinois Agricultural Experiment Station [28] reported that 111 samples of colors from commercial ice-cream plants had counts from

0 to 14 million per milliliter and 12.6 per cent contained *Escherichia-Aerobacter* organisms. Tracy[23] found that organisms grow in color solutions over a wide temperature range. He suggested that color solutions be made with water at 82.2 C (180 F) and held in clean sterile bottles. When not in use, the bottles should be covered and stored at 4.4 C (40 F). Measuring graduates should be washed and rinsed with hot water (82.2 C) each day before use. Color solutions should be prepared weekly; old solutions should be heated at 62.8 C (145 F) for 30 min before use.

BACTERIA FROM CONTAMINATION INCIDENT TO MANUFACTURE AND HANDLING

Contamination incident to the manufacture and handling of ice cream comes from (1) utensils and equipment, (2) employees, and (3) air.

Utensils and equipment. Unsterilized utensils and equipment are as important a source of bacteria with ice cream as with milk and cream. In an ice-cream plant, cans, vats, coolers, etc., must be considered in this connection, but homogenizers and freezers are especially serious sources of organisms because of their construction.

Contamination from homogenizers may be extensive unless proper methods of treatment are employed. The organisms gradually are washed out of a homogenizer so that the first material through the machine is the most heavily contaminated.

Hammer and Goss[27] studied contamination from a freezer by adding sterile water and then operating it for about the freezing period, after which the bacterial content of the water was determined; the freezer was being carefully washed after use but not before the next operation. In five trials the bacterial counts per milliliter of added water were: 3,700; 141,500; 8,050; 1,195; and 300. The bacterial increase with an ice-cream mix would have been more than with the water because the latter did not hold air and larger volumes were required to fill the freezer as full as an ice-cream mix would have filled it. Fay[28] reported an instance in which, under plant conditions, the mix before being placed in the freezer contained 26,000 bacteria per gram, whereas after freezing it contained 625,000; he considered that part of the increase may have been due to breaking up of clumps of organisms but that most of it probably was the result of a poorly washed freezer.

Dahlberg and Marquardt[29] treated freezers with chlorine solution, hot water, and steam. The chlorine solution did not penetrate the bearings and bacteria subsequently developed in the wet freezer. The refrigerant in the freezer chilled hot water so that excessive amounts at very high temperatures were required to sterilize. Heating by steam was slow; but a freezer could be sterilized satisfactorily with steam and such treatment was particularly effective as it dried the freezer and sterilized the bearings. A chlorine rinse prior to using a freezer was considered desirable as a supplement to steam sterilization.

Hot water, steam, and chlorine for sanitizing batch ice-cream freezers were compared by Fabian *et al.,*[30] the freezers previously having been cleaned by treating with cold water, then with warm water (60 to 65.6 C, 140 to 150 F), containing washing powder, and finally with hot water (82.2 C, 180 F). The first rinse water caused the greatest reduction in numbers of bacteria in the freezer and each subsequent washing reduced the number but to a lesser degree. There was a direct relationship between the volume of water used to rinse a freezer and the number of bacteria remaining in it. For sanitizing the front and rear bearings of a freezer, the order of effectiveness was steam, chlorine, and hot water; the rear bearing was harder to sanitize than the front bearing. As judged by the bacterial content of sterile rinse water, chlorine was the best sanitizing agent, steam next best, and hot water least efficient. In general, the preliminary treatment given a freezer was more important than the method used to sanitize it, since adequate rinsing reduces the bacterial load to a point where sanitizing is greatly facilitated. Shutting off the liquid ammonia during rinsing and sanitizing limits the dissipation of heat.

Employees. The employees in an ice-cream plant are a source of organisms in the finished product. Extent of such contamination depends primarily on the personal habits of the individuals involved. Organisms coming from human sources are especially objectionable because of the possibility of pathogens being included and, with the modern methods of making ice cream, carriers and cases of disease among the plant employees constitute the most important source of such organisms.

Air. Contamination of dairy products from the air in plants ordinarily is small, although without special air treatment some organisms regularly come from this source. Fabian [31] investigated contamination from the air in an ice-cream plant and concluded that it was insignificant. The majority of the bacteria present in the air were peptonizers, alkali formers, or inert types; there were a few weak acid formers but practically no strong acid formers. Fewer molds than bacteria were found. Factors increasing the organisms in the air, in what was considered their order of importance, were, (1) dry and windy weather, (2) open doors and windows, (3) dry floors, and (4) machinery in operation. Fabian noted that the amount of air ordinarily entering a freezer during its operation was small and that a normal overrun could be obtained with all the openings to the freezer closed air tight.

CHANGES IN NUMBERS OF BACTERIA DURING MANUFACTURE OF ICE CREAM

A number of the processes used in the manufacture of ice cream influence the bacterial content. Some of them cause decreases but others commonly result in increases, as determined by the plate method, because of the breaking up of clumps of organisms.

Making the mix. Hammer and Sanders[32] noted that an ice-cream mix may contain a smaller number of bacteria per milliliter, as determined by the plate method, than the cream used in preparing it. In eight trials the bacterial content of the mix was 8 to 75 per cent (avg 43.6%) lower than that of the cream from which it was made. The smaller number of bacteria in the mix than in the cream may be due, in part, to a volume change, as a result of adding other ingredients to the cream, but extent of the decrease in certain instances is such that other factors must be operative. One of these undoubtedly is the plasmolyzing action of the sugar that makes up from 13 to 18 per cent of the ordinary mix. This plasmolyzing action apparently varies with different species of organisms so that a significant decrease in bacterial count when a mix is made cannot always be expected and it is probable that with some mixes no decrease occurs.

Pasteurizing the mix. Pasteurization of a mix commonly results in a high destruction of bacteria. Hammer and Sanders,[32] using exposures of 61.1 to 65.6 C (142 to 150 F) for 20 min, found that in nine trials counts after pasteurization ranged from 170 to 66,000 per milliliter, whereas before pasteurization they ranged from 56,000 to 15,300,000; extensive destruction of bacteria had occurred by the time the pasteurization temperature was reached. The investigators suggested that the high concentration of sugar in the mix may influence the pasteurization efficiency obtained. In 46 trials, Fabian and Cromley[33] noted that pasteurization at 65.6 C for 30 min destroyed 94.5 to 99.9 per cent of the organisms in the mix with an average of 98.97 per cent; after pasteurization the counts ranged from 200 to 50,000 per milliliter, whereas before pasteurization they varied from 50,000 to 5,200,000. In comparisons made by Fay and Olson[34] the mix before pasteurization averaged 17,261,926 per gram; after heating, usually to about 65.6 C for 30 min, the counts averaged 219,953. The average bacterial efficiency was 98.69 per cent and the extremes 86.10 and 99.99.

Dubois and Martin [35] compared the effects of various pasteurization exposures on the bacterial flora of ice-cream mixes, using 62.8 C (145 F) for 20, 25, and 30 min, 65.6 C for 20, 25, and 30 min, 71.1 C (160 F) for 10, 15, and 20 min, 76.7 C (170 F) for 2, 5, and 10 min, and 82.2 C (180 F) flash. In the first trials the pasteurization was carried out in test tubes but, later, quantities of mix were pasteurized under plant conditions. Differences in bacterial destruction by the various exposures were too small to be measurable by the plate method. In mixes made from pasteurized dairy products the flora after pasteurization consisted largely of slow acid formers, together with some peptonizers, alkali formers, and inert types; in mixes made from raw dairy products it consisted of peptonizers, alkali formers, and inert types.

Dowd and Anderson [36] found that pasteurization at 82.2 C for 19 sec proved as effective in reducing the number of bacteria in ice-cream mix as 71.1 C for 30 min. The average plate count of the mix prior to pasteurization was 305,000 per milliliter, with HTST pasteurization after homogenization it was 28, with HTST pasteurization before homogenization it was 36, with LTLT pasteurization after homogenization it was 52, and with LTLT pasteurization before homogenization it was 188.

It appears that an ice-cream mix tends to protect certain organisms during pasteurization. Fabian and Coulter [37] found that ice-cream mix has a greater protective action than skim milk for *Escherichia-Aerobacter* organisms when heated at 60 or 62.8 C (140 or 145 F) for 30 min. None of the ingredients of ice cream alone had such a protective effect. Fay [38] concluded that addition of sugar to ice-cream mix may increase the thermal resistance of the microflora. Hypertonic solutions of dextrose and sucrose made up in broth, milk, water, or ice-cream mix afforded certain cells definitely greater protection against heat than when heated in water suspensions. He suggested that certain sugars retard the agglomerating action of heat on the protoplasmic colloids and as a result cells subjected to a given heat treatment in concentrated sugar solutions are only reversibly coagulated, whereas in aqueous suspensions the protoplasm more closely approaches the irreversible stage of coagulation. In comparative studies on milk and ice cream, Paley and Isaacs [39] found that the latter exerts a protective action for *E. coli* against a pasteurization temperature of 61.7 C (143 F), the apparent resistance of the organism being approximately doubled. The effect was attributed to stabilizers (locust bean gum and sodium alginate); sugar and gelatin exerted no protective action.

On the basis of the heat resistance of an especially resistant strain of *E. coli,* Myers and Sorensen[40] suggested that a pasteurization temperature of not less than 65.6 C (150 F) and preferably 68.3 C (155 F) be maintained for 30 min in order to ensure destruction of the colon group of bacteria in ice-cream mix.

There is evidence that the solids in ice cream also protect phosphatase against destruction by exposures that destroy it in milk.[41,42] This relationship should be considered in applying the phosphatase test to ice cream.

Pasteurization exposures used with ice-cream mixes vary widely. There is a definite tendency to employ comparatively high exposures to ensure a pronounced destruction of organisms, and such exposures are readily applied to ice-cream mix.

Homogenizing the mix. Homogenizing a mix usually results in an increase in bacterial content, as determined by the plate method. This may be due, in part, to contamination from the machine but breaking up of clumps of organisms also is a factor.

Fabian and Cromley[33] made counts on mixes after pasteurization and again after homogenization at 2,500 to 3,000 lb pressure. In 74.0 per cent of the 46 comparisons there was an increase ranging from 5.9 to 1,400 per cent, in 10.8 per cent there was a decrease ranging from 9.1 to 86.2 per cent, and in 15.2 per cent there was no change. Fay and Olson[34] found the average bacterial contents in 28 trials were 219,953 per gram before homogenization and 277,475 after. There was an increase in count in 18 trials, a decrease in 9, and no change in 1; considering all the counts, there was an increase of 26 per cent as a result of homogenization. Peterson and Tracy,[43] in a study of the condensation process of preparing an ice-cream mix, noted that in the 2 trials investigated homogenization increased the count.

Aging the mix. From the data available on storage of milk and cream it would be expected that a properly cooled mix could be aged for a reasonable period without significant increase in organisms. Fay and Olson[34] obtained data indicating that, in general, aging a mix 16 to 24 hr does not markedly affect the bacterial count when the temperature is below 7.2 C (45 F); the average count on 28 mixes before aging was 191,782 per gram and after aging 192,362. A mix made by the condensation process was held at 0 to 1.7 C (32 to 35 F) by Peterson and Tracy;[43] the count originally was 1,400 per milliliter, after 5 days it was 1,700, after 14 days 762,000, after 23 days 42,210,000, and after

32 days 188,500,000. The results show the bacterial development that can be expected in mixes held for excessive periods at temperatures permitting growth of organisms.

The studies of Mueller [44] indicated that a short aging period at a relatively high temperature, followed by aging at a low temperature, increased the basic viscosity and gel strength of both a gelatin ice-cream mixture and a pure gelatin water solution, when compared with aging at a low temperature only. Mueller and Frandsen [45] then compared aging ice-cream mixes at 20 C (68 F) and 3.3 C (38 F); the mixes were pasteurized at 62.8 C (145 F) for 30 min and homogenized at this temperature, using 2,500 lb pressure. Aging at 20 C for 2, 4, or 6 hr had no measurable effect on the bacterial content. Mueller and France [46] found that aging pasteurized ice-cream mixes for 6 hr at 20 C, whether followed by aging for 18 hr at 3.3 C or not, gave no significant increase in bacterial content; when aged 24 hr at 20 C a definite increase did not occur until about the tenth hour. Aging an unpasteurized mix for 6 hr at 20 C, whether followed by aging for 18 hr at 3.3 C or not, gave an increase in bacterial content. Aging of mixes for 24 hr at 3.3 C did not give an increase in bacterial contents.

Nelson [47] reported that the bacterial count of ice-cream mix stored at 4.4 C (40 F), or above, increases with storage and may reach considerable magnitude as the storage temperature increases. Coliform bacteria usually increased in numbers in ice-cream mix at 8 C (46.4 F), or above; the increases may lead to false conclusions relative to post-pasteurization contamination based upon presence of coliform organisms in appreciable numbers.

Freezing the mix. Freezing a mix commonly results in an increase in the bacterial content, as determined by the plate method, even when the condition of the freezer is such that it is not a significant source of organisms. In 51 comparisons of the bacteria in the mix and in the frozen but unhardened ice cream, Hammer and Goss [27] found that in 43 (84.3%) the count was increased from 2 to 227 per cent (avg 46.3%), in 6 (11.8%) it was decreased from 2 to 31 per cent (avg 13.0%), whereas in 2 (4.0%) there was no change. Ellenberger [6] noted an average increase of 48 per cent in the number of bacteria as a result of freezing. Fabian and Cromley [33] made 41 comparisons of the counts before and after freezing; in 46.3 per cent there was an increase, as a result of the freezing, varying from 0.95 to 260 per cent (avg 46.4%); in 36.6 per cent there was a decrease ranging from 3.85 to 59.7 per cent (avg 25.95%); in 17.1 per cent there was no change. In 28 trials Fay and Olson [34] found the average count on the mix was 192,362 bacteria per gram and on the frozen ice cream it was 236,688; in only 6 of the comparisons was a decrease noted and 4 of these were small enough to be within the limit of experimental error.

The increase in bacterial content that is so frequently obtained during freezing of an ice-cream mix in a clean freezer is undoubtedly due to breaking up of the clumps of bacteria during the agitation in the freezer and, accordingly, should be regarded as an apparent increase. Lowering of the temperature during freezing would be expected to have a destructive influence on certain of the organisms present and this may account for the rather large decreases sometimes noted. The effect of freezing on the bacterial content of a mix is a combination of two distinct and opposing influences, one tending to increase the plate count and the other to decrease it. Presumably, each of these influences has a variable effect, depending especially on the species of organisms present; since the plate counts are most often increased during freezing, the agitation is the more important.

Hardening ice cream. Hardening ice cream after it comes from the freezer results in a pronounced decrease in its temperature and this would be expected to destroy some of the contained organisms, extent of destruction being dependent on the species present. Hammer and Goss [27] made 52 comparisons of the bacterial content of unhardened and hardened ice cream; in 45 (86.5%) hardening caused decreases ranging from 2 to 75 per cent (avg 39.1%), in 6 (11.5%) it caused increases varying from 7 to 22 per cent (avg 13.8%), whereas in 1 (1.9%) there was no change. The few increases probably were due to experimental error since there was no opportunity for addition of organisms. In 28 comparisons Fay and Olson [34] found that the average bacterial content before hardening was 236,688 per gram and after 2 or 3 days in storage it was 186,320; increases occurred in 8 of the trials. The investigators consider that the increases were due to experimental error.

Storage of ice cream. Ice cream ordinarily is stored at temperatures from somewhat below −17.8 C (0 F) to somewhat above. Under these conditions growth of micro-organisms would not be expected. Hammer [19] reported bacterial counts that showed that there is a decrease or very little change in numbers of bacteria in ice cream during storage; he also pointed out certain objections to data reported in the literature which showed an increase. Esten and Mason [48] concluded from a study of 12 samples that, when ice cream is kept frozen for periods of at least 1 mo, there is no marked increase or decrease in the bacteria, as shown by litmus lactose gelatin plates. Hammer and Goss [27] studied 39 samples of ice cream held by packing in ice and salt and 12 held in a commercial hardening room and found no evidence of an increase in numbers of contained bacteria, whereas commonly there was a decrease. Ellenberger[6] noted that there is no radical change in total numbers of bacteria in ice cream during storage. He also found that the groups of bacteria in ice cream, as determined by litmus gelatin plates and litmus milk tubes, do not change noticeably during storage. Fabian and Cromley[38] concluded that storage of ice cream at an average of −20.5 C (−4.9 F) for 1 day to 3 weeks decreases the plate count in the majority of cases although there may be variations in individual samples.

Hammer and Goss[27] reported that softening and rehardening ice cream may result in either a significant increase or decrease in the number of bacteria present, the effect presumably being dependent on the species of bacteria and the extent of the softening. An increase seemed to be more common in ice cream that was only slightly softened, whereas a decrease followed pronounced softening, probably because of the more destructive action of the rehardening. Large increases in the bacterial content of ice cream resulting from softening and rehardening are important if a bacterial standard is being enforced.

Manufacture of ice cream with low bacterial count. Use of methods that tend to yield ice cream with low bacterial counts is now widespread among well-managed ice-cream plants. High-quality ingredients are carefully handled in properly sterilized equipment, with just as little opportunity for contamination from human sources as possible, so that a clean and safe product can be marketed. Pasteurizing and homogenizing the entire mix, with the exception of certain ingredients whose bacteriological quality can be maintained satisfactorily, has advantages from a sanitary standpoint. There is relatively little handling after the heating and, accordingly, little opportunity for contamination from utensils, employees, etc.

Attempts to produce ice cream with low bacterial plate counts often result in values of only a few thousand per milliliter or, occasionally, under 1,000. Such low values are possible only with efficient pasteurization and adequate protection against recontamination. The factor over which the manufacturer has the least control is the quality of the cream and other dairy products and when these contain many bacteria that are resistant to the pasteurization exposures counts are relatively high. Unless proper methods are used, cutting and wrapping of bricks and slices, preparation of individual molds, etc., may result in contamination of the product because of the special handling involved.

CONTAMINATION OF ICE CREAM BY VENDORS

Ice cream is extensively consumed in public eating establishments of various types under conditions that necessitate its being dipped from relatively large containers. The dishes used are possible sources of contamination but, because of the way in which dippers and scoops ordinarily are held, they appear to be especially important in this connection.

Krog and Dougherty [49] took samples of the same bulk ice cream with the vendor's scoop and also with a sterile spoon from a freshly exposed surface after removal of at least 1 in. of surface ice cream with another sterile spoon. Bacterial counts invariably were higher on the scoop samples, and often the difference was striking. Water in which the scoops were kept showed counts from 4,000 to 6,336,000 per milliliter. With better care of the scoop, including rinsing in hot or cold running water before and after each use, contamination from the scoops was largely eliminated. A survey by Fabian and Hook [50] of 96 establishments retailing ice cream indicated the total bacterial count, as well as the coliform count, was lower when running water instead of still water was used in dipper receptacles. The speed at which the water was running through the receptacles also was of importance.

Abele [51] found that bacterial counts on water in which ice-cream dippers were immersed ranged from 600 to 20,000,000 per milliliter, with some plates too heavily seeded to count. The count on water said to be changed every other day or so was 6,900,000 per milliliter; the average (logarithmic) count on 5 samples said to be changed once daily was 2,400,000, that on 4 samples said to be changed twice daily was 79,000, that on water said to be changed 10 times daily was 3,500,000, and that on water said to be changed 15 times daily was 210,000. The average count on water in 5 receptacles into which a small stream of water was constantly flowing was 9,500 per milliliter (from 600 to 77,000). Blair [52] reported ice-cream scoops were being immersed between use in very turbid water, having bacterial counts of 540,000 and 297,000 per milliliter, respectively.

With the Burri technic, dipper contamination can be studied by comparing numbers of organisms at the interior and surface of dipped portions of ice cream. Surfaces of some of the samples encountered by Long and Hammer [10] yielded strikingly larger numbers of colonies than the interiors.

When ice cream is dipped for consumption elsewhere, the container may be an additional source of contamination. Speck and Black[53] found the bacterial counts of open-top ice-cream containers obtained from retail establishments varied widely; they apparently were correlated with the storage conditions in the stores. Many of the containers yielded more than 1 organism per milliliter of capacity. Contamination from air and dust probably was the most important factor influencing the counts. Typical organisms contaminating the paper containers belonged to the genera *Micrococcus, Alcaligenes, Sarcina, Bacillus,* and *Achromobacter.*

BACTERIAL STANDARDS

Bacterial standards commonly are used in the control of ice cream supplies. They vary rather widely. The usual standards are easily met when good ingredients and suitable manufacturing procedures are employed. Pasteurization of the entire mix at relatively high temperatures is helpful in this respect. Occasional lots of cream may contain many spore-forming or non-spore-forming heat-resistant bacteria so that the pasteurization is not as efficient as usual.

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Chapter 14

Bacteriology of Lactic Acid Cultures

Cultures of micro-organisms in milk or other materials frequently are inoculated into dairy products to improve the quality. The practice apparently began with use of sour milk to hasten acid formation in sweet milk and gradually developed until cultures of selected organisms now are used as an aid in the production of the desired flavor and acidity in various dairy products and in the control of objectionable fermentations, especially in certain cheeses.

The cultures most widely employed in the dairy industry are the lactic acid cultures, or starters. They are characterized by rapid production of lactic acid and by flavor development when grown in milk or cream at favorable temperatures. Normally, two distinct types of bacteria are present, one producing primarily lactic acid from lactose and one fermenting citric acid with formation of various compounds, at least some of which are important from the flavor standpoint.

Lactic acid cultures frequently are used in butter plants, where they may be referred to as butter cultures. Although many plants, particularly in certain parts of the world, make butter without use of cultures, other plants find them very beneficial. They give butter a high flavor that is essential in many markets, they tend to mask certain off flavors, especially feed flavors and those absorbed by the cream, and they tend to prevent development of certain bacterial defects in butter, although not to the extent that these defects are entirely controlled. In the manufacture of unsalted butter for table use, cultures are necessary since this product must have a high flavor to be satisfactory.

In the cheese industry lactic acid cultures are widely used to give the desired acid increases during manufacture and/or ripening of various cheeses. Commonly, in cheese manufacture the most important quality in a culture is rapid acid production, and the flavor compounds formed in the fermentation of citric acid are of relatively little importance; accordingly, pure cultures of lactic acid bacteria sometimes are employed with satisfactory results.

Lactic acid cultures also are used in preparing culture buttermilk and in manufacture of margarine. With both these products flavor is an important consideration, just as in buttermaking, and the culture employed often is selected on the basis of flavor.

Early history. The first attempts to control flavor development in cream intended for churning consisted of addition of clean-flavored sour milk or buttermilk from a churning of good butter. In 1889, Conn [1] began publication of a series of reports dealing with the ripening of cream. He early recognized that the process is a complex one and questioned the ability of any one organism to produce what is known as ripened cream. Also, he [2] pointed out that in cream ripening something besides souring takes place. Conn studied the influence of various organisms on the quality of butter. Of 20 species whose effects were reported in 1893,[3] only 3 produced strikingly bad results, whereas the others gave good butter or had no marked influence; in no case did a single species produce typical ripening and the few acid-producing types did not have so good an influence as the alkali formers. In 1896, Conn [4] pointed out that acid and flavor should be distinguished, and that although the acid is developed from lactose the flavor probably comes from some other source.

Use of cultures of selected bacteria for the ripening of cream was the result of the work of Storch (Knudsen).[5] He studied organisms from such sources as buttermilk, butter, and ripened cream and found variations in the flavors produced by different acid-forming types. Among seven such organisms isolated from ripened cream obtained at a plant known for the high flavor of its butter, there was one that yielded as full an aroma as ripened cream possesses; in view of the present information, this culture may have included the type of organism now recognized as largely responsible for the desirable aroma of lactic acid cultures. Storch supplied the cultures used to inoculate the heated cream when the first trials on pasteurization of cream intended for butter were carried out in 1888. Although the cultures did not yield such fine-flavored butter as that produced by inoculating buttermilk from a churning of good butter, souring defects were avoided.

Weigmann [6] also found variations in the products formed by acid-producing bacteria. When there was a full aroma the butter did not have a clean flavor or good keeping quality, and when there were both a clean flavor and good keeping quality the butter lacked aroma. Accordingly, this investigator suggested the use of mixtures of organisms or an attempt to find cultures which combined the two qualities.

Commercial cultures for cream ripening appear to have become available in Europe in 1890. Their use increased rapidly, especially in Denmark; in 1897,[7] out of 866 butter plants in that country, 802 used cultures. Although the superiority of Danish butter at that time commonly was attributed to use of cultures, the influence of pasteurization of the cream also was recognized. In the United States commercial cultures were studied, especially by Conn,[8] who pointed out that they were not all alike and that, although most were pure cultures, some were mixtures; one culture contained no less than 10 or 12 species.

More recent history. Following the early studies it commonly was believed that lactic acid cultures are pure cultures of lactic acid bacteria. There was little evidence for this and some to the contrary since both Conn and Weigmann had failed to isolate an organism that would bring about the changes occurring in ripening cream. In 1919, various investigators noted the presence of an additional type of organism in normal lactic acid cultures. Hammer and Bailey [9] pointed out that lactic acid cultures produce considerable volatile acid in milk, whereas *S. lactis* does not, and reported the isolation from them of organisms that yielded considerable volatile acid and evidently were important in flavor development. Storch [10] noted the presence in lactic acid cultures of organisms that he called *x* bacteria and Boekhout and Ott de Vries [11] described their "aroma" bacteria. More recently various investigators have found that proper flavor development in lactic acid cultures requires action of two distinct types of organisms. Recognition of this relationship has emphasized the complexity of the fermentation occurring in the cultures and explained why cultures showing a normal rate of acid production often lack flavor.

Hammer [12] reported that citric acid is the primary source of the volatile acid formed in lactic acid cultures by the organisms associated with *S. lactis*. Various observations have confirmed this and also indicated that citric acid is the source of a number of other compounds of importance.

In 1929, the studies of van Niel *et al.*[13] and of Schmalfuss and Barthmeyer [14] showed the importance of biacetyl as an aroma constituent of butter and indicated that it is formed through oxidation of acetylmethylcarbinol. Biacetyl ($CH_3-CO-CO-CH_3$) is a very volatile, yellow liquid with a sharp pungent odor. When sufficiently dilute, the odor suggests the odor of butter. Acetylmethylcarbinol ($CH_3-CHOH-CO-CH_3$) is a liquid that readily polymerizes to a solid form, especially at low temperatures; the polymerized form often persists at ordinary temperatures. Highly purified acetylmethylcarbinol is odorless but the usual preparations have an odor more or less suggestive of dilute biacetyl. When present in dairy products the two compounds often have been determined together by oxidizing the carbinol to biacetyl with ferric chloride, distilling this off, along with the biacetyl originally present, and precipitating as nickel dimethylglyoximate. The values can be expressed as the weight of nickel salt, or can be calculated to biacetyl or acetylmethylcarbinol. More recently colorimetric methods have been used to determine the carbinol and/or biacetyl.

The importance of acetylmethylcarbinol plus biacetyl in lactic acid cultures was shown by Michaelian *et al*,[15] who found that cultures having a satisfactory flavor contained comparatively large amounts of these materials, whereas cultures lacking in flavor contained relatively small amounts or none. The following data illustrate this relationship:

MILLIGRAMS OF NICKEL DIMETHYLGLYOXIMATE (EQUIVALENT TO ACETYLMETHYLCARBINOL PLUS BIACETYL PER 200 GM CULTURE) FROM SATISFACTORY CULTURES AND CULTURES LACKING FLAVOR

	Satisfactory Cultures	Cultures Lacking Flavor
No. cultures examined	51	41
No. cultures yielding 0.0 mg Ni salt	0	16
No. cultures yielding 0.1 to 4.9 mg Ni salt	0	20
No. cultures yielding 5.0 to 9.9 mg Ni salt	0	5
No. cultures yielding 10.0 to 19.9 mg Ni salt	20	0
No. cultures yielding 20.0 to 29.9 mg Ni salt	21	0
No. cultures yielding 30.0 to 39.9 mg Ni salt	10	0
Maximum amount of Ni salt	39.5 mg	7.4 mg

Milk cultures of *S. lactis,* or of the organisms associated with it in lactic acid cultures, ordinarily do not contain significant amounts of acetylmethylcarbinol or biacetyl so that the relatively large amounts of these compounds in lactic acid cultures are the result of associative action of the two types, the *p*H produced by *S. lactis* greatly influencing the products formed by the other type.

STREPTOCOCCI ASSOCIATED WITH *S. LACTIS* IN LACTIC ACID CULTURES

General characters. The organisms normally associated with *S. lactis* in lactic acid cultures are typical streptococci. In a lactic acid culture they are not distinguishable from *S. lactis* microscopically; in pure cultures they often are somewhat smaller than *S. lactis*. The organisms grow poorly on artificial media; standard, tomato, beef infusion, and whey agars are relatively satisfactory for them. Orla-Jensen *et al.*[16] reported the favorable influence of yeast extract, even in small amounts, on the growth and noted that it stimulated acid production from various fermentable materials.

The outstanding character of the organisms is their ability to ferment citric acid. The principal products formed from this acid are acetic acid, carbon dioxide, and a series of neutral C_4 compounds which include acetylmethylcarbinol, biacetyl, and 2,3-butylene glycol ($CH_3-CHOH-CHOH-CH_3$). The proportion in which the C_4 compounds are produced varies widely with the conditions under which the fermentation occurs, particularly with the *p*H. Biacetyl and acetylmethylcarbinol accumulate in significant amounts only when the *p*H is relatively low.

Although the organisms associated with *S. lactis* in cultures produce carbon dioxide in milk, the gas production is inconspicuous, and cultures may be studied in a routine way without any gas being noted. Sealing of freshly inoculated milk cultures, for example with a mixture of paraffin and Vaseline, tends to retain the gas formed, and the seal is forced. The gas production of these organisms also can be shown by inoculating cans of evaporated milk and soldering the opening through which the inoculation was made; the gas developed ordinarily results in bulging of the cans. An occasional culture rapidly coagulates milk, and this coagulation may be accompanied by the presence of definite bubbles of gas.

Nomenclature. The citric-acid-fermenting streptococci normally present in lactic acid cultures have been divided into two species, *Streptococcus citrovorus* and *Streptococcus paracitrovorus*.[12] The fundamental difference between them is that *S. citrovorus* produces no lactic acid in milk, whereas *S. paracitrovorus* forms a variable amount of *l* lactic acid. Because of this difference *S. citrovorus* does not redden litmus milk, except on extended incubation, and produces a comparatively low volatile acidity; but *S. paracitrovorus* reddens litmus milk and produces a comparatively high volatile acidity, the lactic acid tending to make the citric acid more available. The distinction between the two species is not a sharp one, since the amount of acid produced by *S. paracitrovorus* is so variable. In a study of 124 cultures of *S. paracitrovorus* Hammer and Baker [17] found the total acidities produced in milk varied from 0.27 to 0.99 per cent (avg 0.46), whereas the volatile acidities * ranged from 12.5 to 40.0 (avg 28.6) without added citric acid and from 44.4 to 96.6 (avg 73.9) with 0.4 per cent citric acid added to the milk. Some of the cultures coagulated milk; others did not. With an occasional culture, coagulation was rapid.

Knudsen and Sorensen [18] designated the organisms *Betacoccus cremoris;* Hucker [19] considered that they belong to the genus *Leuconostoc* and recognized two species, *Leuconostoc citrovorus* and *Leuconostoc dextranicus.* The citric-acid-fermenting streptococci characteristic of lactic acid cultures are conveniently designated the flavor organisms.

Isolation. Isolation of the flavor organisms from lactic acid culture, or comparable material, ordinarily can be accomplished by plating. The plates are poured with a suitable medium, such as tomato agar, incubated at 21.1 C (70 F), and well-developed colonies picked into litmus milk. Cultures that do not show the typical *S. lactis* reaction are examined for morphology and the general changes produced in milk, including the fermentation products. The comparatively small number of the flavor organisms in lactic acid culture and the uneven distribution complicate their isolation by direct plating. In some cases many colonies include none of these organisms but, occasionally, most of the colonies picked are of this type.

By inoculating lactic acid culture on an agar slope and carrying it through a number of transfers on such a medium, with several days between transfers, cultures sometimes are obtained that do not show an *S. lactis* reaction when inoculated into litmus milk. Occasionally, lactic acid culture which has been held for a considerable period so that many of the contained organisms are dead reaches a condition in which it yields growth on an agar slope but fails to give an *S. lactis* reaction in litmus milk. Cultures obtained by either of these methods can be plated to establish the purity and the final selections studied for their general characters.

Benchetrit [20] found that tomato agar modified by addition of a small amount of α-bromopropionic acid is very useful in isolating flavor organisms from lactic acid culture and other materials. The acid is added to the melted and cooled agar just before pouring and the inoculum is either distributed through the medium or smeared on prepared plates. The concentration of acid necessary to inhibit *S. lactis* varies with different lots of tomato agar but usually ranges from 0.2 to 0.6 ml of *N*/10 solution per 100 ml of agar. With the correct concentration of acid, growth of *S. lactis* is rather effectively checked without seriously influencing growth of the flavor organisms; various bacteria forming large colonies also grow on the medium.

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* The volatile acid in a culture is determined by steam-distilling a mixture of 250 gm of culture with 250 ml of distilled water, after addition of 15 ml $N/1$ sulfuric acid, and titrating the first 1,000 ml of distillate, using $N/10$ sodium hydroxide and phenolphthalein; distillation is at such a rate that approximately 2 hr are required to obtain the 1,000 ml. Results are expressed as the milliliters of $N/10$ sodium hydroxide required. Only a portion of the volatile acid is obtained with this procedure so that the method is primarily of value for comparative purposes.

Distribution. The flavor organisms regularly are present in lactic acid cultures of satisfactory quality. *S. paracitrovorus* has been repeatedly obtained from sour milk and cream, but *S. citrovorus* is much less common in these materials. Both species sometimes are obtained from butter, in which case they probably represent organisms derived from the culture used. Certain of the organisms isolated from various cheeses are citric-acid-fermenting streptococci.

In some instances in which pure cultures of *S. lactis* have been carried through a series of transfers in butter plants, the cultures eventually have had a typical culture flavor. Undoubtedly, this has been due to contamination in the plants and indicates that the flavor organisms are widely distributed. It also explains why cultures of *S. lactis* can be sold as lactic acid cultures and satisfactory results obtained in a certain percentage of cases.

COMPOUNDS FORMED DURING RIPENING OF LACTIC ACID CULTURES

Ripening of a lactic acid culture involves a series of chemical changes that are the result of the activity of the two types of bacteria normally present, and certain of the compounds formed account for the characteristic flavor of the culture (Babel and Hammer).[21]

Lactic acid. The compound produced in largest amount during ripening of a culture is lactic acid. A ripened culture contains 0.75 to 1.00 per cent or more acid, calculated as lactic (*p*H 4.3 to 4.7). Lactic acid cultures vary considerably in the rate of acid production because of differences in the strains of organisms present and other factors. Although lactic acid is odorless, it has a definite influence on the flavor because of its acid taste. The lactic acid is formed largely from lactose by *S. lactis* and, accordingly, is the *d* form. However, Hammer [22] noted that ripened cultures contained some *i* lactic acid, the percentage being variable when *S. citrovorus* was associated with *S. lactis* in the culture and generally high when *S. paracitrovorus* was present. *S. paracitrovorus* definitely produces *l* lactic acid from lactose.

It is probable that the lactic acid in cultures tends to inhibit certain contaminating organisms and prevents development of defects by them. Spore-forming bacteria commonly survive pasteurization of the culture milk, but rarely multiply significantly because of their sensitivity to acid.

Volatile acids. Considerable amounts of volatile acids are produced in a culture. These acids influence the flavor of the product and account for the distinct acid odor. Hammer and Sherwood [23] found that the volatile acids contained in a highly ripened culture were made up largely of acetic acid with a small amount of propionic, and in a culture ripened to a low acidity a greater percentage of the volatile acid was propionic. It appears that the small amount of volatile acid produced by *S. lactis* from lactose is acetic with considerable propionic, whereas the much greater amount of volatile acid formed by the flavor organisms from citric acid is largely acetic. The volatile acid formed in a lactic acid culture is greatly increased by adding citric acid to the milk used.

Carbon dioxide. Carbon dioxide is produced in a culture in relatively small quantities, most of it being formed by the flavor organisms. Ordinarily, the carbon dioxide remains in solution or diffuses into the air although, occasionally, a few bubbles are noted in a coagulated culture. If citric acid is added to the milk used, the carbon dioxide formed is greatly increased; through supersaturation of the fermenting milk, the carbon dioxide may be largely held in solution until the culture is stirred or otherwise agitated, when bubbles form rapidly. Carbon dioxide has an influence on the flavor of a culture and the effect is increased with citric acid added to the culture milk.

C_4 compounds. It is generally agreed that the C_4 compounds in a lactic acid culture are produced primarily by the flavor organisms from citric acid and that the combined yield of the compounds can be greatly increased by adding citric acid to the milk. However, there is a difference of opinion with reference to the mechanism involved. According to one idea, acetylmethylcarbinol is the primary compound formed; at a high *p*H it rapidly is reduced to 2,3-butylene glycol, whereas at a low *p*H it tends to accumulate and some of it is oxidized to biacetyl. According to another idea, acetic acid and carbon dioxide are produced from citric acid at a high *p*H and the C_4 compounds are formed only at a relatively low *p*H; acetylmethylcarbinol or biacetyl may be formed, depending on the oxygen supply, and then under certain conditions either one may be reduced to 2,3-butylene glycol.

Acetylmethylcarbinol. It appears that acetylmethylcarbinol is important in a culture primarily because large amounts of it tend to be accompanied by comparatively large amounts of biacetyl, although the actual quantities of biacetyl are much the smaller. Prill and Hammer [24] indicated that such factors as oxygen supply, temperature, content of citric acid, etc., prevent the establishment of any close relationship between the two compounds. Acetylmethylcarbinol is the intermediate C_4 compound from the standpoint of oxidation. Its rapid reduction to 2,3-butylene glycol under favorable growth conditions for the flavor organisms prevents its accumulation in a culture until the *p*H is comparatively low. Apparently, certain strains of *S. lactis* produce small amounts of acetylmethylcarbinol from lactose and thus may be a minor factor in its production in a culture; under certain conditions, they also tend to reduce it.

Biacetyl. Biacetyl is the important C_4 compound in a culture because it largely accounts for the pleasing neutral flavor and in the preparation of certain dairy products in which a culture is used development of biacetyl is the primary consideration. Biacetyl is not limited to lactic acid cultures and dairy products in which culture is used but is found in a variety of materials,[21] including raspberry juice, strawberry juice, honey, roasted coffee, bread, cane molasses, tobacco smoke, etc.; acetylmethylcarbinol also is found in various materials.

Although acetylpropionyl has an odor resembling that of biacetyl, lactic acid cultures apparently contain neither higher homologs of biacetyl nor methylglyoxal; [25] also, homologs of acetylmethylcarbinol are absent.

2,3-butylene glycol. Apparently, 2,3-butylene glycol accumulates in a culture as a result of the reduction of acetylmethylcarbinol and biacetyl, primarily by the flavor organisms. Reduction is more rapid under conditions that favor activity of the organisms, such as a suitable temperature and low acidity. This explains the more rapid disappearance of the carbinol and biacetyl in a culture at 21.1 C (70 F) than at lower temperatures and also the relatively rapid decrease in a neutralized culture. The glycol is the final C_4 compound formed; in a culture it apparently never is oxidized to the carbinol or biacetyl.

Conditions favoring production of acetylmethylcarbinol and biacetyl. Active production of acetylmethylcarbinol and biacetyl by the flavor organisms requires a satisfactory *p*H. This can be established by other acids as well as by lactic acid. Michaelian *et al.*[26] added different acids in different amounts to pure cultures of the organisms, incubated them 48 hr at 21 C (69.8 F), and determined the acetylmethylcarbinol plus biacetyl. In general, the yield with lactic acid as the acidulant was comparatively low and a significant production occurred over a rather narrow *p*H range, maximum yields being obtained from *p*H 4.4 to 4.1. Sulfuric acid gave higher yields than lactic acid and production was significant over a wider *p*H range, with maximum yields at *p*H 3.6 to 3.2. A mixture of 0.15 per cent lactic acid and variable amounts of sulfuric acid gave yields comparable to sulfuric acid alone, with maximum yields at *p*H 3.9 to 3.5. With citric acid the yields were much higher than with other acids and a significant production occurred over a wide *p*H range, with maximum yields at *p*H 3.9 to 3.7. The general results indicate that other acids are more satisfactory than lactic for the establishment of conditions favoring production of acetylmethylcarbinol and biacetyl by the flavor organisms.

The biacetyl production of the flavor organisms in milk was investigated by Cox [27] at *p*H values from 5.5 to 4.4, these being obtained by adding lactic acid. The organisms produced biacetyl and then partially or wholly destroyed it. The amounts produced varied with the different strains and the production capacity could not be correlated with any other property of a strain. Rate of growth of the organisms was progressively slower the lower the *p*H but at least as much biacetyl eventually was produced at a low as at a high *p*H. The biacetyl-producing power per unit cell appeared to increase with decreasing *p*H.

A low *p*H apparently makes the citric acid in milk more readily available to the flavor organism. Michaelian and Hammer [28] noted that addition of lactic acid to milk cultures of *S. citrovorus* or *S. paracitrovorus* increased the volatile acid production. The effect varied greatly with different strains; it was greatest with those producing little change in the titrable acidity of milk and least with those producing a conspicuous change. Such acids as tartaric, phosphoric, and sulfuric had the same effect as lactic.

Addition of citric acid to milk for cultures regularly increases the production of acetylmethylcarbinol and biacetyl during the ripening, other factors being satisfactory. Laboratory results and plant experience indicate that during the ripening of a culture the flavor organisms can ferment more citric acid than normally is present in milk.

The oxygen supply is important in the production of biacetyl. Michaelian and Hammer [29] found that its production in acidified skim milk by pure cultures of the flavor organisms definitely was influenced by bubbling various gases through the freshly acidified cultures; oxygen regularly gave a higher yield of biacetyl than the control, through which no gas was bubbled; carbon dioxide, hydrogen, or nitrogen gave lower yields. The gases had the same general effect on production of biacetyl in lactic acid cultures as in the pure cultures, but the actual quantities of biacetyl formed appeared to be smaller, which may have been due to the smaller number of citric-acid-fermenting organisms per milliliter. Addition of purified acetylmethylcarbinol to sterile skim milk adjusted to an acidity satisfactory for rapid production of the carbinol by the flavor organisms did not yield appreciable amounts of biacetyl in 48 to 72 hr, either with or without treatment with gas, which indicates the formation of biacetyl from acetylmethylcarbinol in a culture is not a direct chemical oxidation but results from the action of organisms.

Brewer *et al.*[30] found that production of biacetyl in lactic acid cultures, and in pure cultures of the flavor organisms in milk, was greatly increased by growing the cultures under 30 lb, or more, air pressure per square inch, provided the cultures were agitated by aeration or stirring. The increase frequently amounted to several hundred per cent. In some cases the yield of acetylmethylcarbinol was increased and in other cases it was not. Pressure without agitation did not increase the biacetyl content. The relatively high biacetyl contents of the aerated-pressure cultures were evident in the flavor of the butter in which the cultures were used.

Prill and Hammer [24] found that various factors tended to give a relatively high biacetyl content in cultures. Addition of citric acid was very important. Shaking ripened cultures produced significant increases and lack of aeration caused decreases. Holding ripened cultures at various temperatures gave increases but holding at low temperatures was apparently the most practical. Addition of as little as 0.08 per cent citric acid to a ripened culture gave increases, the agitation incident to the distribution presumably also being a factor. Lowering the *p*H of a ripened culture, even with such an acid as sulfuric, tended to increase the biacetyl content. In general, a high acetylmethylcarbinol content was favored by similar conditions but attainment of a relatively high content at low temperatures was slower than with biacetyl.

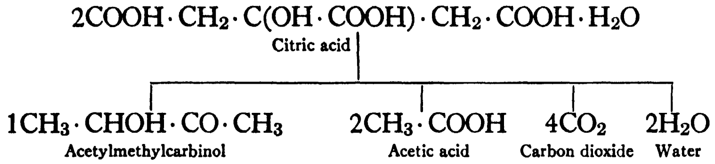
General consideration of flavor development in cultures. Ripening lactic acid cultures regularly lack flavor until the *p*H is rather low and then in a surprisingly short period they have considerable flavor. Barnicoat [31] reported that with a commercial culture the amount of acetylmethylcarbinol plus biacetyl at 0.75 per cent acid was only about one-third of that at 0.81.

Since the flavor organisms are so necessary in producing the desirable compounds in a culture, their number at the time the proper *p*H is established is extremely important. If the number is too low, a culture lacks flavor. An adequate number must be built up during the early part of the ripening period so that when the proper *p*H is reached formation of biacetyl can occur quickly. On the other hand, an excessive number early in the ripening period is objectionable since the organisms may destroy so much of the citric acid under conditions which do not favor accumulation of biacetyl that too little remains to be fermented at a desirable *p*H. A rather delicate balance between the types of bacteria normally in a culture is very necessary for the most desirable flavor development. Hoecker and Hammer [32] noted that addition of lactic acid to milk cultures of the flavor organisms resulted in larger amounts of biacetyl than were present in lactic acid cultures; the comparative numbers of flavor organisms per milliliter of the two types of cultures may have been a factor in this increase.

At a satisfactory *p*H and temperature, production of acetylmethylcarbinol and biacetyl appears to continue as long as citric acid is present. By addition of sufficient citric acid to the original milk, the amounts of these compounds that can be formed are surprisingly large and the odor of biacetyl then is very conspicuous.

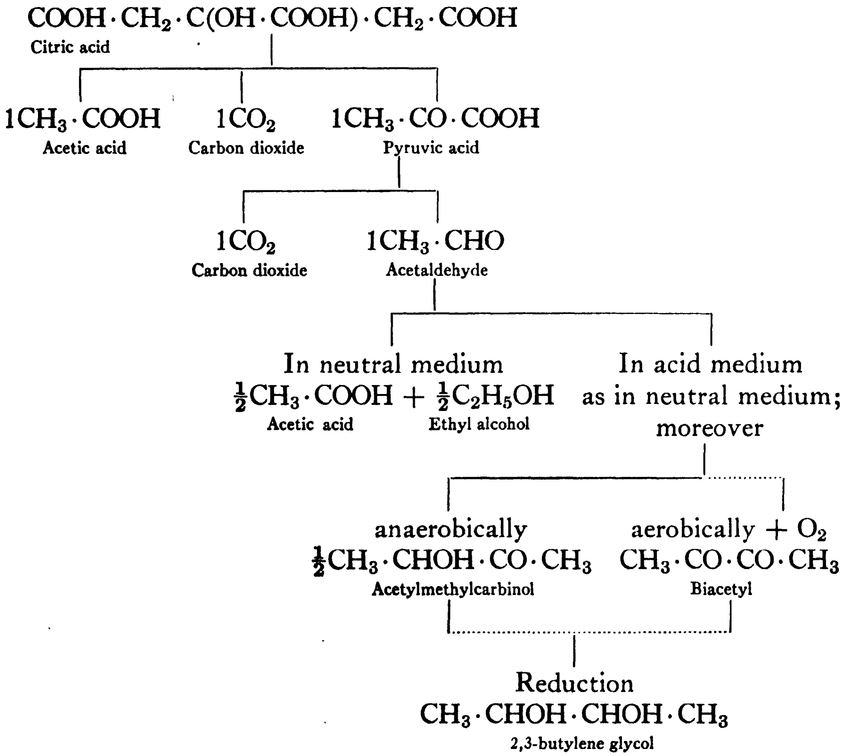
A desirable flavor that has developed in a culture is not maintained indefinitely. It appears that as soon as the maximum amounts of acetylmethylcarbinol and biacetyl are produced, their reduction begins, the rate being dependent on various factors. Since the reduction is due primarily to activity of the flavor organisms, it can be delayed by use of low temperatures and it is a common practice to cool ripened cultures and hold them at relatively low temperatures. With citric acid still present, cooled culture may continue to increase in acetylmethylcarbinol and biacetyl for some time. Certain butter plants find it advisable to hold ripened cultures cold for about 24 hr before use to permit an increase in biacetyl. Such an increase is made more certain by adding a small amount of citric acid to the culture at the time cooling is begun. Maintenance of a high biacetyl content in a ripened culture is especially important when the culture is to be added to cream at a low temperature to avoid growth of the culture organisms in the cream. Apparently, both lactic and volatile acids are not destroyed by the normal culture organisms.

Breakdown of citric acid. Rumments[33] suggested that the breakdown of citric acid by the flavor organisms with the formation of various compounds is as follows:



The carbinol then can be oxidized to biacetyl or reduced to 2,3-butylene glycol, depending on conditions.

A more elaborate scheme for the breakdown of citric acid, in which a distinction is made between the breakdown in neutral and in acid reaction, was proposed by van Beynum and Pette[34] and is as follows:



Protein breakdown. Barthel and Sadler[35] reported that when lactic acid cultures were inoculated into sterile milk containing calcium carbonate and incubated 2 months at room temperature under hydrogen or carbon dioxide, there was an increase in both the soluble and amino nitrogen. With the soluble nitrogen the increase was essentially the same as that produced by the lactic acid streptococci, but with the amino nitrogen it was much greater. Anderegg and Hammer[36] found that lactic acid cultures regularly proteolyzed milk, whereas some *S. lactis* cultures did and others did not. When calcium carbonate was added to the milk and occasionally distributed by agitation, proteolysis was more pronounced than when it was not added. With *S. lactis,* an increase in soluble nitrogen was usually accompanied by an increase in amino nitrogen. *S. citrovorus* and *S. paracitrovorus* did not proteolyze milk. Hammer and Patil[37] reported that proteolysis was evident in freshly coagulated lactic acid cultures grown in pasteurized or sterilized milk. On extended holding, proteolysis was more pronounced when calcium carbonate was added to the milk than when it was not added. They also noted that *S. citrovorus* and *S. paracitrovorus* did not definitely proteolyze milk. Kelly[38] investigated the proteolytic action of *S. lactis* and *S. cremoris* in milk, with and without calcium carbonate. The carbonate increased the action of the organisms on protein and sugar. Little difference was noted in the rate of production or in the final amount of acetic acid-, trichloroacetic acid-, or phosphotungstic acid-soluble nitrogen produced by the two species.

Products of the protein breakdown in lactic acid cultures probably have little direct effect on the flavor but they may favor growth of *S. citrovorus* and *S. paracitrovorus.* Also, desirable protein degradation in certain cheeses may be due in part to the culture organisms.

PROPAGATION OF LACTIC ACID CULTURES

Preparation of lactic acid cultures is one of the very important operations in any plant using them since cultures of good quality so definitely contribute to the quality of the products in which they are employed. In making high-scoring culture butter, quality of the culture is exceeded in importance only by quality of the cream and poor culture actually may be harmful; if contaminating organisms are present, they sometimes include species which cause deterioration in butter. In cheesemaking, use of unsatisfactory culture may result in off flavors and if the culture is slow in producing acid the manufacturing operation may be greatly delayed. In culture buttermilk, flavor and body are so directly related to action of the culture used that without good culture the final product is certain to be unsatisfactory.

Success in the preparation of cultures requires attention to many details. When the steps are carried out hurriedly, high-quality cultures cannot be obtained regularly. Variation in the quality of the cultures results in variation in the quality of the products made with them and this is very undesirable from the marketing standpoint. Care of the cultures should have precedence over all other plant operations and should not be left until there is nothing else that needs attention. If the ripening is to be properly controlled, conditions must be uniform from day to day, and when the procedures have once been standardized for a particular plant the only variable factor should be the amount of inoculation. It is especially important to inoculate the cultures at a definite time each day because intelligent variations in the amounts of inoculation can be made only when the ripening period is rather definite.

Natural lactic acid cultures. At one time cultures that were called natural starters were obtained by allowing selected raw milk or cream to develop acid and then transferring this material in pasteurized milk. A common procedure was to permit a number of samples to sour and then employ the one with the best flavor. Buttermilk from a churning of good butter also was used to develop cultures. In some instances satisfactory natural cultures were obtained but the chances of securing a good culture in this way are small. Sometimes natural cultures are satisfactory for a number of transfers and then suddenly develop off flavors; this is due to increased activity of objectionable organisms that, for a time, have been controlled by the desirable species.

Commercial lactic acid cultures. Various laboratories supply lactic acid cultures that are more or less carefully selected by means of bacteriological methods and these are called commercial cultures. They are supplied in either liquid or powder form.

The usual liquid employed for commercial cultures is milk, although bouillons and other materials also have been used. Commonly, milk is sterilized in bottles and then inoculated and shipped as requests for cultures are received. Coagulation may occur during transportation of the culture; if it does not, the culture is allowed to stand after it is received and is used for inoculation when coagulation occurs. Another procedure is to transfer, under aseptic conditions, small portions of developed lactic acid culture to the bottles used for transportation. This method permits the culture to grow at a favorable temperature, instead of the varied temperatures encountered duing transportation, but there is a disadvantage in the longer exposure of the organisms to a relatively high acidity.

Powdered cultures have been prepared from good lactic acid cultures by adding an inert material, such as starch, lactose, milk powder, etc., to absorb a part of the water and then drying at a low temperature. Special drying procedures also may be used. The handling necessary in the preparation of a dry culture may introduce foreign bacteria, but under the usual conditions of propagation these are gradually eliminated when the desired species begin active multiplication. The drying kills many of the organisms but those left are in an inactive condition and remain so because of lack of moisture; they survive for long periods, much longer than if they could produce excessive acidities.

The advantage of liquid cultures is that the organisms are active and yield a satisfactory culture very soon; their disadvantage is that they have poor keeping qualities because the organisms are in a medium in which growth can continue until the products formed influence their activity. Powdered cultures have good keeping qualities, since the organisms are dormant, but this inactive state results in growth starting slowly and a number of transfers may be necessary to get the cultures in a satisfactory condition.

In shipping milk cultures of various organisms that actively produce acid, it is a common practice, when the distances are great, to add a small amount of sterile calcium carbonate. This procedure has been found useful with lactic acid cultures, especially when the temperatures during shipment are not above 30 C (86 F). Because of the liberation of gas by reaction of the carbonate and the acid in the culture, magnesium oxide also has been employed but the organisms do not appear to survive so well as with calcium carbonate. Pittman [39] reported use of agar containing peptone, lactose, calcium carbonate, and tricalcium phosphate for shipment of cultures to cheese factories.

Destruction of organisms during drying. Rogers [40] suggested that exposure to a high concentration of salts during the preparation of powdered cultures is largely responsible for the destruction of organisms. From his data showing the percentage of water and the bacteria per gram at intervals during the drying of a culture, it appears that at a water content between 5 and 10 per cent there was a pronounced decrease in the numbers of bacteria. Rogers had cultures dried by a process in which the water was removed from the milk by a spray carried up by a current of warm, dry air; drying was complete in a short time and took place at a low temperature. Powders prepared from cultures of an active lactic acid species grown in milk showed large numbers of organisms per gram, although there was a pronounced decrease from the numbers that would have been present had there been no destruction of organisms. Data on 11 powders showed counts varying from 657,500,000 to 8,590,000,000 per gram.

A method of drying cultures by exposing them in a frozen condition over sulfuric acid in a vacuum approximating 0.01 mm was studied by Rogers. Powders prepared in this way were so active they curdled milk in 17 hr at 30 C (86 F) when added in the ratio of 1 part to 1,000,000 parts of milk. The numbers of bacteria in 11 powders varied from 380,000,000 to 12,670,000,000 per gram.

The investigations of Rogers established a number of significant points with reference to powdered cultures. Addition of dibasic potassium phosphate increased the total numbers of bacteria in a milk culture but the powder made from such a culture was less active than one made from milk without the addition. Activity of a powder was not increased by neutralizing the culture with calcium carbonate before drying. Loss of activity in a powder was very slow at 0 C (32 F), or lower, and became more rapid as the temperature increased until at 30 to 37 C (86 to 98.6 F) dried cultures of lactic acid bacteria became inactive in a short time. When the moisture content was comparatively high, loss of activity in powders was more rapid than when it was low. Cultures held in a vacuum retained their activity much better than cultures in an atmosphere of nitrogen or hydrogen, and the most rapid loss of activity occurred in an atmosphere of oxygen or air.

The use of the low-temperature, high-vacuum, drying technic has been applied to the commercial production of lactic acid cultures.[41]

Holding commercial cultures. In the holding of coagulated liquid, or powdered, commercial cultures in plants before they are inoculated, low temperatures should be used. Although powdered cultures can be kept much longer than liquid cultures, low temperatures, such as 0 C (32 F), materially increase their life, just as with liquid cultures. Even with the organisms in a dormant condition, death is materially delayed by low temperatures.

Mother cultures. Ordinarily, lactic acid cultures are carried in small quantities in milk under well-controlled conditions to provide mother cultures that can be employed to prepare bulk cultures for use in dairy products. They should have special attention in an attempt to keep them vigorous and uniform, with good flavor, body, and texture. A defect in a bulk culture involves only the operations of one day, whereas a defect in a mother culture may be repeated in succeeding transfers. Ordinarily, a number of mother cultures are carried so that comparisons can be made and one of satisfactory quality selected.

Mother cultures are carried in various types of containers, such as milk bottles, glass fruit jars, glass-stoppered bottles, special flasks closed with waterproof paper, etc. Containers of materials other than glass are not advisable as they limit observations and, if metal is exposed to the culture, it may be attacked by the acid. Small-mouth containers have the advantage of offering only a limited area through which material from the air can enter. Approximately the same amount of milk should be used in the containers from day to day since this aids in controlling the rate of inoculation.

Milk for cultures should be of high quality, both as to flavor and bacterial count. Although certain off flavors are largely eliminated by pasteurization or are masked by the heated flavor developed, many of them carry through into the ripened culture and are objectionable. On the average, milk with a high bacterial count cannot be so completely freed of organisms as milk with a low count and, occasionally, organisms that resisted the pasteurization are responsible for abnormal conditions in cultures; moreover, excessive numbers of organisms in the milk may cause an abnormal flavor.

The milk can be pasteurized and then added to sterilized containers but such handling after heating affords an opportunity for contamination, and it is preferable to pasteurize the milk in the containers in which it is to be used. A satisfactory procedure is to place the containers, to which the milk has been added, in water reaching above the level of the milk and then heat the water with steam or in some other way. A metal box with a perforated false bottom and a cover is very useful in carrying out this method; the size is determined by the number of mother cultures and the type of container used for them. The milk should reach 82.2 to 93.3 C (180 to 200 F) and be held there for at least 30 min. It is then cooled by slowly running water into the water around the containers, care being taken to lower the temperature gradually so as to prevent breakage; slow cooling is especially necessary if the containers have thick walls. The milk should be cooled to 21.1 or 22.2 C (70 or 72 F) and a commercial culture added and thoroughly distributed. Powdered cultures, with which coagulation sometimes is slow, are often incubated at temperatures above those usually used for cultures to bring about more rapid coagulation. Acid development is not the only factor involved, however, and there is the possibility, with this procedure, that one of the desirable species in the culture will be overgrown. The temperature of the inoculated milk should be held as constant as possible. As soon as a mother culture is firmly coagulated it should be cooled, unless it is to be used for inoculation at once, in order to delay further action of the contained organisms.

A second mother culture is developed from the first by inoculating a small portion into milk prepared in the same way as that used for the original culture. The inoculation should be made under such conditions that contamination will not occur. Pouring over the lip of the container is not satisfactory because of the danger of adding foreign organisms and the difficulty of obtaining a definite idea of the amount of inoculating material. A satisfactory method is to use glass tubes or pipettes that have been heated with boiling water, steam, or dry heat; with these there is little chance of contamination, inoculations can be kept uniform or varied in any way that appears desirable, and cultures can be tasted by drawing small amounts into the mouth.

The series of mother cultures begun in this way is continued as long as the quality is satisfactory. Inoculations commonly are made daily, but a day can be missed occasionally without objectionable effects, if the ripened cultures are kept cold. Some plants, particularly those using relatively small amounts of culture, have found it possible to limit the transferring of mother cultures to three, or even two, times a week and still obtain satisfactory results.

Dahlberg and Ferris[42] found that, when a lactic acid culture was inoculated every day and also every third day and carried under excellent conditions, the quality was identical, as judged by appearance, flavor, and acid development. However, the freshly coagulated culture was somewhat less satisfactory in cheesemaking.

Amount of inoculating material. The amount of inoculating material to use is so variable that definite statements with reference to it cannot be made. It depends on the ripening period, activity of the culture, accuracy of the temperature control, and various other factors. The common ripening period is from afternoon until the next morning (14 to 18 hr), although in some instances mother cultures have been ripened during the day (6 to 9 hr); the latter method requires very heavy inoculations. With the longer ripening, the usual procedure is to inoculate the second lot of pasteurized milk with from 0.25 to 1 per cent of the first mother culture. From then on the amount is varied on the basis of the degree of ripening. Changes are made frequently as the culture appears to be underripe or overripe, and for this reason approximate measurements of the inoculating material are necessary. The rate of ripening should be controlled entirely by the amount of inoculation and not by varying the temperature.

Controlling temperature of ripening mother cultures. Various methods of maintaining, or attempting to maintain, satisfactory ripening temperatures are used with mother cultures. At the one extreme is the system of placing the inoculated milk in the location where the temperature is least objectionable; for example, the boiler room in winter and an open window on a warm summer night. At the other extreme are the automatically operated controllers that are heated with electricity and cooled with ice or brine. There are various simple systems of heating an insulated cabinet with an electric light or other resistance that are satisfactory; in summer, when room temperature is higher than the temperature desired, the cabinet can be held in the refrigerator. In many instances a well-insulated container, such as an

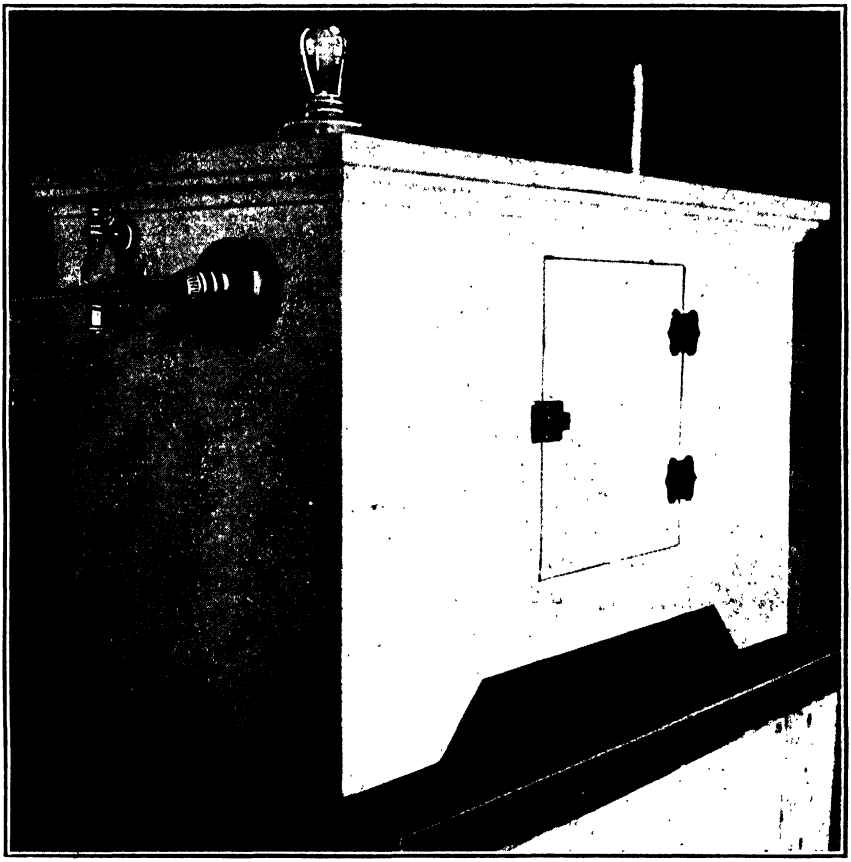


Fig. 28. Homemade temperature controller for mother cultures.

ice-cream cabinet or a specially made box without heat, has proved satisfactory for holding mother cultures, especially when they are surrounded with a large volume of water to delay temperature changes.

In preparing mother cultures it is essential to have a temperature controller, the exact type to be determined by the funds available. Homemade equipment often is very satisfactory and the efficiency is dependent principally on the ingenuity of the builder. The question of sanitation should be considered so that the equipment can be kept free of odors coming from wet wood or insulating material. A metal lining for the holding compartment is advisable and necessary if a volume of water is used to delay temperature changes; the water should be replaced frequently to avoid development of odors. Wilster and Price[43] devised a tank in which milk for mother cultures could be pasteurized and cooled, and the inoculated milk then incubated.

Cooling cultures after ripening. Definite coagulation is the usual basis on which a culture is cooled to limit action of the contained organisms. It can be determined without opening the container and thus without danger of contamination. Considerable acid must be present in a culture before the desired flavor is developed, and at low acidities cultures regularly lack flavor. Cordes and Hammer[44] noted that at low acidities the percentage of total acid made up of volatile acid also was low and, as the total acid increased, this percentage increased. High production of acetylmethylcarbinol plus biacetyl does not occur until a relatively high acidity has developed. On the other hand, after a good development of the carbinol plus biacetyl has occurred there is a destruction of these materials. When a culture is examined for flavor a decision as to whether it is overripe or underripe should be made and, on the basis of this, the inoculation of later transfers can be adjusted. At present, cultures commonly are ripened to higher acidities than formerly.

Overripening. Moderate overripening apparently does not influence the ability of a culture to coagulate rapidly milk into which it is inoculated; Hammer and Baker[45] found that more than 30 hr holding at 21.1 C (70 F) after coagulation did not appreciably decrease the rate at which cultures coagulated milk, as compared to the rate at the time of curdling. In some cases cultures which have lost their ability to develop a full flavor can be improved by definite overripening. Since the citric-acid-fermenting streptococci are rather tolerant of acid, continued ripening may result in these organisms making up a larger percentage of the total flora and thus giving more flavor in the later transfers. Overripening is the simplest method of attempting to improve cultures that lack flavor; however, extended overripening may seriously influence the activity of the organisms.

Golding *et al.*[46] reported that the acidity of fresh cultures in the range usually found in a cheese factory had little effect on the rate of acid development in skim milk and that refrigeration for 24 hr did not change the activity of a culture when grown at the approximate optimum temperature. Johns and Bérard[47] found that severe and prolonged overripening of cultures to an extent greater than usually encountered in cheese-factory practice failed to slow down the rate ot growth or acid development, or to lower the final acidity reached. An overripened portion of a culture worked slightly faster in a cheese vat and produced cheese with a higher flavor score than the control. In two of three cultures studied, the overripened portion contained more rapid-coagulating organisms than the normally ripened portion. After 30 days of repeated overripening, the flavor of the overripened portions was superior to that of the normally ripened portions.

Preparation of large volumes of lactic acid culture. Large volumes of lactic acid culture are prepared with the same general procedure that is employed for mother cultures. Commonly, the milk is pasteurized in culture tanks or vats equipped with steam and cooling water, and the ripening carried out in these. Some plant operators prefer to ripen culture in 10-gal cans. These can be placed in a tank of water and the heating, cooling, and incubation of the milk carried out in the tank by controlling the temperature of the water, or the milk can be heated and cooled in a vat and then transferred to sterilized cans that are held in a tank of water to control the ripening temperature. The inoculation of a large volume of milk is made directly with the required number of mother cultures or a special intermediate culture for the inoculation can be prepared in a container of suitable size. The amount of inoculating material is more difficult to determine with a large volume of milk than with a mother culture because of variations in the quantity from day to day and greater fluctuations in ripening temperature.

Contamination of cultures. Cultures sometimes contain organisms other than the normal species. These may have gained entrance during the propagation in the plants or they may have been present in the cultures from the distributing laboratories. Walts[48] examined nine cultures (seven liquid and two powdered) from various manufacturers and found five of them contaminated, four with alkali-forming bacteria, and one with yeasts. Microbiological examinations of cultures being carried under plant conditions indicate that contaminating organisms rather frequently are present, usually in small numbers but occasionally in large numbers. Ordinarily, the contamination is not evident from the flavor of the culture; in other cases it is.

The organisms contaminating cultures include bacteria, yeasts, and molds. In some cases foreign bacteria can be eliminated from a culture by exposing them to high acidities through overripening, but such a procedure is not effective with certain organisms, especially yeasts and molds, because of their acid tolerance. The mold most frequently found in cultures is *O. lactis.* In some cases it is so numerous it can be seen in many fields of a microscopic preparation; under these conditions products made with the culture are heavily contaminated.

Holding cultures. Frequently it is desirable to hold cultures for extended periods in order to have a reserve supply of satisfactory inoculating material. This can be done conveniently by freezing the cultures and maintaining them at a temperature that prevents thawing. In many cases cultures that have been frozen for as long as 2 mo still are very active when inoculated into freshly pasteurized milk and incubated at 21.1 C (70 F). The cultures are frozen in small quantities to prevent breakage of the containers.

JUDGING LACTIC ACID CULTURES

Anyone propagating lactic acid cultures should have the ability to evaluate their general quality. Both the desirable and undesirable characters should be noted each time a culture is examined. An understanding of the probable causes of various objectionable conditions is essential if the quality of the cultures is to be maintained.

General appearance. Ripened mother cultures can be examined for their general appearance at any time but the remainder of the examination should be delayed until transfers have been made because of the possibility of contamination. If bubbles of gas have formed they usually are present near the bottom of a culture although sometimes they are distributed throughout. Furrows made by bubbles passing to the surface sometimes are evident next to the wall of the container. Since *S. citrovorus* and *S. paracitrovorus* produce carbon dioxide from citric acid, a normal lactic acid culture occasionally shows a few gas bubbles because of the activity of these organisms. Gas-forming contaminants commonly give an objectionable flavor to a culture as well as produce gas bubbles in it. In some cases these organisms can be eliminated by a series of transfers but the process is uncertain and a contaminated culture should be discarded unless the culture was at one time exceptionally good, when the normal culture organisms can be isolated and recombined. Whey at the surface of a culture is of no great significance. It may be due to high acidity, although often there is high acidity without the presence of whey. If a culture is agitated, even slightly, at about the time of coagulation, whey frequently collects.

Texture, body, and flavor. When a culture is to be examined for texture, body, and flavor, it first is thoroughly broken up by shaking or stirring. A small amount then is poured or dipped into a cup or other container and the body and texture quickly judged. The texture should be smooth and creamy, without any suggestion of lumps; there undoubtedly are various causes for an uneven texture but one that has been definitely established is an incubation temperature that is much too high. The body should be of such a character that when the culture is poured on a surface it stands up well and has a velvety appearance. A thin body may be due to low acidity but sometimes is caused by contaminating organisms. A culture may show ropiness, and when this occurs *S. lactis* var. *hollandicus* or a ropy strain of *S. citrovorus* or *S. paracitrovorus* ordinarily can be isolated. With some cultures ropiness appears and disappears for no apparent reason. Although pronounced ropiness in thoroughly ripened culture definitely is objectionable, slight ropiness that quickly disappears on agitation is not important. Some cultures show conspicuous ropiness at low acidities and little or none when ripe. Occasionally, a culture has a dull, chalk-white appearance; the common cause of this is overripening.

The flavor of a culture should be delicate and pleasing, and free from any suggestion of an objectionable condition. If a culture is insufficiently ripened it will lack flavor; if overripened the flavor will not be delicate but coarse. The decrease in acetylmethylcarbinol plus biacetyl content of a culture with extended overripening is reflected in the flavor. There is no definite acidity, which always gives the most desirable flavor, and some cultures must be ripened more than others to develop the desired condition; with a given culture there also may be variations from day to day. In some instances a culture lacks flavor because the citric-acid-fermenting organisms develop poorly, or not at all, and under these conditions no amount of ripening will give the desired flavor.

Abnormal flavors. A number of objectionable flavors, such as barny, unclean, bitter, yeasty, rancid, etc., are encountered in cultures. These are the result either of some condition of the original milk or the activity of undesirable organisms during the ripening. Selection of the milk on the basis of freedom from off flavors is very essential if a good product is to be obtained. Contaminating organisms that cause objectionable flavors in cultures come from various sources, such as unsterile transfer equipment or contamination from the mouth of the container, and when once present in a culture may be carried along from one transfer to the next. Differences in extent of their growth from day to day result in variations in intensity of the condition caused by them; the condition may even seem to disappear entirely and then later be very evident.

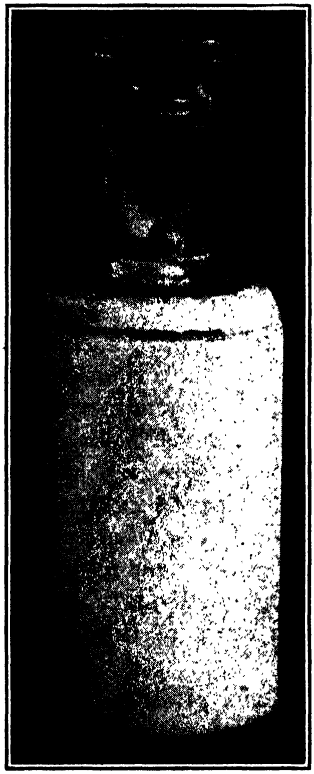


FIG. 29. Mother culture showing whey beneath the cream layer.

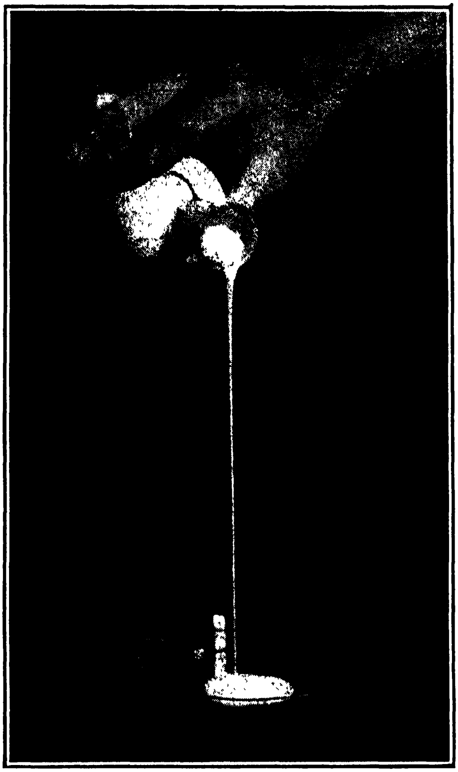


FIG. 30. Ropy mother culture.

Conditions to observe in judging cultures. In judging the flavors of cultures it is essential that the temperature be approximately uniform from day to day if accurate comparisons are to be made. Anyone accustomed to examining cultures at fairly low temperatures has difficulty in judging those that have not been cooled. When a culture is poured into a glass or cup the flavor should be judged at once since free contact with air for only a few minutes may have a striking influence on it. There is a distinct advantage in having a number of cultures to compare and they should differ in quality. When only one culture is examined day after day it is difficult to recognize small but significant defects, and often the culture is considered to be better than comparisons would show it to be. Another advantage is that several cultures offer more assurance of having satisfactory inoculating material since it is improbable that all the cultures will develop defects at the same time unless there has been some irregularity in preparation, such as improper pasteurization of the milk or unsuitable incubation temperatures.

GENERAL CONSIDERATIONS IN PREPARING LACTIC ACID CULTURES

Many factors have an influence on the quality and general condition of lactic acid cultures. Some of these have been studied and their effects recognized so that they can be considered in propagating cultures.

Influence of the milk. Both whole milk and skim milk are widely used in the preparation of cultures, comparative costs often being the determining factor in the selection. The fat is not involved in the fermentation but gives the product a full flavor that is as pleasing in culture as in sweet milk. Barnicoat[31] investigated the effect of the medium on development of acetylmethylcarbinol and biacetyl. Results obtained when milk or cream was steamed 30 to 40 min, allowed to cool, and inoculated with 0.5 per cent culture indicated there was no essential difference, when due allowance was made for the fat content of the cream, in the action of the culture in skim milk, whole milk, or cream. Milk reconstituted from evaporated milk ordinarily is not satisfactory for cultures; commonly, coagulation is slow, the curd is soft, and the conspicuous heated flavor is objectionable. Milk powder that reconstitutes well, whether from skim milk or whole milk, often makes satisfactory culture. In the preparation of culture buttermilk in areas where milk is in short supply, fresh milk sometimes is extended by mixing with it milk reconstituted from powder.

Using a uniform rate of inoculation and period of incubation with lots of milk from individual animals, Baker and Hammer[49] noted that variations occurred in the acidities produced by a given culture. There was a tendency for the milk from an animal to maintain the same general ranking as to the acidity produced; in general, milk having a high content of total solids gave a high acidity when it was made into culture but the correlation was not a close one. Considerable variation also occurred in the flavors and the milk from an animal fluctuated from time to time in the quality of the cultures prepared from it; these differences may have been due, in part, to variations in acidities of the different cultures. Milk from various herds also showed differences in the acidities developed under definite inoculation and incubation conditions but these were less than the differences in milk from individual animals.

Kelly[50] carried cultures in certified milk from a certain source and found that a flat, yeasty flavor regularly developed. When milk from another herd was employed the cultures developed a satisfactory flavor. Two lactic acid strains isolated from a culture made with the certified milk produced the flat, yeasty flavor when grown in the certified milk, but gave a clean flavor after the first few transfers in milk from the other source.

Knudsen and Sorensen[18] reported that in certain cases milk from individual cows would not produce a usable culture. This sometimes was true when no disease could be detected in the animals, even on post-mortem examination, but more commonly a thorough study showed a disturbed secretion that usually was caused by an udder infection. The investigators considered that the property of milk which is important in producing a satisfactory culture is the buffer action. When they obtained poor cultures the buffer action was low and the milk might be either normal or abnormal in other respects. However, they noted that in some abnormal milk there are objectionable effects other than buffer action.

Watson[51] found that Jersey milk has a markedly greater buffer capacity than Holstein milk in the *p*H zone of 5 to 6. This may explain why milk high in solids sometimes is preferred for propagating cultures. Watson also noted that pasturing the cows had a negligible effect on the buffer value of milk.

Results of various investigations indicate that mastitis milk is unsatisfactory for cultures. Davis[52] noted that such milk has an inhibitory effect on culture organisms which pasteurization partially neutralizes; milk of late lactation has a similar effect. In studies on the growth responses of *S. lactis* in mastitis milk, Prouty[53] used milk from individual infected and non-infected quarters of the same cows. Milk with an initial *p*H higher than 6.9 usually failed to support active growth of *S. lactis*, whereas normal milk from the other quarters permitted normal acid development. Addition of as little as 10 per cent very abnormal mastitis milk to normal milk had a retarding effect on *S. lactis*. Adjusting the *p*H of mastitis milk to that of normal milk only partially improved the acid development; with most of the mastitis samples, heating at 65.5 to 68.3 C (150 to 155 F) for 30 min had little or no effect on acid development by *S. lactis*. Rice[54] reported that in mastitis milk development of the lactic acid streptococci sometimes was strongly checked and even after pasteurization such milk still may prevent normal acid formation; rate of fermentation was slower in late lactation milk than in full lactation milk and was slightly more rapid in milk to which colostrum was added to the extent of 10 to 25 per cent than in normal milk.

Undoubtedly, cultures frequently are made successfully from milk that includes the product of abnormal udders. Irregularities in the effect of mastitis milk on cultures would be expected because of variations in the effect of udder infections on the general composition of the milk secreted.

In many plants milk for mother cultures regularly is taken from a supply that has been satisfactory over an extended period. When difficulties are experienced with the cultures, one of the first steps is to use milk from a different supply. This occasionally corrects the condition but more often it does not, which suggests that selected herd milk commonly is satisfactory for cultures.

Influence of pasteurization exposure. The pasteurization exposure used with milk to be made into culture may influence the rate of acid development. Hammer and Baker[45] found that, when cultures were made from lots of milk pasteurized with different exposures, the milk heated to 62.8 C (145 F) for 30 min developed acid and coagulated more slowly than that heated to considerably higher temperatures. There was essentially the same rate of acid production when the milk was heated to 71.1 C (160 F) for 30 min as when higher temperatures were used for this period. The variations noted in the rate of coagulation following different pasteurization exposures may have been due to the effect of heat on the germicidal property of milk. With a heating period of 30 min the influence of the heat appeared to be gradual and spread over a considerable temperature range, instead of occurring completely at a definite temperature. These results indicate that use of high pasteurization exposures with milk intended for cultures has an advantage in addition to destruction of most of the contained organisms.

Rice[54] noted that with the lactic acid streptococci of cultures acid is formed almost as rapidly in milk heated momentarily to 76.7 C (170 F) as at a higher temperature for a longer period but that there is a slower rate of fermentation in milk heated to 62.8 C (145 F) for 30 min and also in unheated fresh milk.

When raw milk was inoculated with culture, Davis[52] obtained less acid development than with milk heated to 73.9 C (165 F) for 30 min. Milk heated to 82.2 C (180 F) by the flash method formed more acid than milk heated at 73.9 C for 30 min, and milk boiled for 24 hr formed more acid than milk heated to 82.2 C by the flash method.

Influence of ripening temperature. Under practical conditions, where the ripening temperature often is variable, satisfactory cultures may be obtained over a considerable temperature range. Toens and Hammer[55] used various temperatures and found that good cultures could be obtained as low as 18 C (64.4 F) and as high as 32 C (89.6 F); at 37 C (98.6 F) the flavor was very unsatisfactory and the fermented milk did not resemble a lactic acid culture, although sufficient acid regularly developed. Temperatures such as 21.1 to 22.2 C (70 to 72 F) have advantages of considerable importance. With definitely lower temperatures growth of the organisms is delayed and it is difficult to obtain rapid ripening, whereas with significantly higher temperatures overripening often occurs and conditions are more favorable for development of heat-resistant organisms that survive pasteurization of the milk. At relatively high temperatures growth of *S. citrovorus* and *S. paracitrovorus* is greatly influenced and a lactic acid culture that has been through a very few transfers at 37 C may no longer contain these organisms.

From the standpoint of rate of acid development, 21.1 to 22.2 C (70 to 72 F) definitely is not the most favorable temperature and in the manufacture of various cheeses, where the rate of increase in acid is important, higher temperatures regularly are used. In studies on three lactic acid cultures, Golding *et al.*[46] found that the maximum acid development in 8 hr took place between 29.4 and 32.2 C (85 and 90 F); development of acid at 25 and 35 C (77 and 95 F) was approximately one-half that at the optimum and at 15.6 and 37.8 C (60 and 100 F) it was insignificant during the 8-hr test. There was considerable difference in the rates of acid development by the three cultures.

Constant and irregular ripening temperatures. Under practical conditions there often is considerable fluctuation in the ripening temperature. Toens and Hammer [55] studied the influence of changing temperature on the quality of cultures in a series of comparisons in which one culture was maintained at a constant temperature and the temperature of the other, which had been similarly prepared, was allowed to fall. The setting temperature used was 32 C (89.6 F); this was known to give satisfactory cultures under the conditions employed and had the advantage of permitting considerable drop in temperature. In 87 comparisons the constant temperature culture was the better in 61 (70%), whereas the fluctuating temperature culture was preferred in 26 (30%). These results suggest that constant temperatures are desirable in propagating cultures.

Influence of length of ripening period. Toens and Hammer [55] compared rapid (6 to 8 hr) and slow (16 to 20 hr) ripening in 25 trials with each of three cultures, the time of coagulation being controlled entirely by varying the amount of inoculation. Although the differences often were small, the rapidly coagulated culture was considered the better in 64 (85%) of the 75 comparisons, whereas the slowly coagulated culture was placed first in 11 (15%); the percentages of comparisons in which the rapidly coagulated culture was preferred were 92, 76, and 88, respectively, for the three cultures.

Influence of air supply. Although there is not much difference in the time of coagulation between the top and bottom of a volume of milk being made into culture, the bottom commonly coagulates first. Settling of the inoculating material may be a factor in this but the air supply also must be considered. Toens and Hammer [55] compared the rate of acid development in thin layers of milk held in cotton-stoppered flasks with the rate in deeper layers held in glass-stoppered bottles, and found that it was more rapid with a restricted than with an abundant air supply. Rice [54] noted that, at a constant temperature, aeration retards development of acid in milk by the lactic acid streptococci. However, acid formation is not the only effect to be considered and an abundant oxygen supply tends to favor production of biacetyl, which normally is very desirable.

Modification of milk for culture. Various modifications of milk used for culture have been suggested, either to reduce the cost of the culture or improve its quality. Baker and Hammer [49] found that addition of lactose, fat, or water yielded culture with lower acidity and less satisfactory flavor than normal milk. Milk ash gave a salty taste that was not objectionable and tended to intensify the desirable flavor.

The importance of citric acid as a source of the flavoring materials in lactic acid culture and the small amount of this compound naturally in milk (0.16 to 0.18%)[56,57] suggest its addition to milk intended for culture. Templeton and Sommer[58] found that addition of citric acid, or its equivalent as sodium citrate, to the milk used for culture increased the volatile acid production about 50 per cent, but the titrable acidity was increased not more than 10 per cent. They[59] also reported that addition of citric acid or sodium citrate to cream, lactic acid culture, or both, tends to produce butter of more desirable flavor than when no citric acid is used. Various investigations on the acetylmethylcarbinol plus biacetyl content of culture show that the amount of these materials is greatly increased when citric acid is added to the milk used and, in agreement with this, the culture has a high flavor. Commonly, persons accustomed to this type of culture definitely prefer it to culture made without added citric acid because of the high flavor.

When citric acid is added to milk intended for culture, an aqueous solution is prepared. Ordinarily, 0.15 per cent of the acid (crystals containing 1 molecule of water) is employed, although occasionally a larger amount is used. The acid is dissolved in a small quantity of water, using heat to aid in the solution and to destroy most of the organisms present. A glass container is advisable since concentrated citric acid attacks various metals. The solution is allowed to cool and is then poured into the milk that has been pasteurized and cooled to the incubation temperature. The acid is added to the milk very slowly and with considerable agitation to prevent curdling the milk at the point where the acid strikes it. After the acid has been thoroughly distributed the milk is inoculated and ripened in the usual way.

It appears that culture with a satisfactory flavor is more regularly obtained when citric acid is added to the milk than when it is not. Ordinarily, the acid is used only in large volumes of culture since its principal effect is to give more flavor, rather than to influence the proportionate numbers of the two types of organisms present.

DEVELOPMENT OF LACTIC ACID CULTURES

With the information available relative to the organisms normally present in lactic acid cultures it is possible to develop new cultures under such conditions that only desirable bacteria are present. The two types of organisms, *S. lactis* and *S. citrovorus* or *S. paracitrovorus*, can be mixed directly in sterile milk or the latter type can be grown for a few hours, or overnight, and *S. lactis* then added. When the mixed culture has developed considerable acid it is carried through a number of transfers in sterile milk and then, when coagulation becomes rapid, it can be used in thoroughly pasteurized milk. All the transfers should be grown at the temperature usually used with cultures. The early transfers may coagulate slowly because of the restraining action of the citric-acid-fermenting type on *S. lactis* and, for this reason, the interval between the inoculation of the two types should not be excessive.

The *S. lactis* culture used should be one that forms acid rather rapidly and does not produce an objectionable flavor. Farmer and Hammer [60] noted that cultures developed with *S. lactis* var. *maltigenes* had a malty flavor through a series of transfers; *S. lactis* strains producing a flavor suggesting cabbage when grown alone in milk developed the same flavor in combination with certain citric-acid-fermenting strains, whereas with others they did not. An *S. lactis* strain which combines satisfactorily with one citric-acid-fermenting strain may not combine satisfactorily with another.

There appears to be no basis on which citric-acid-fermenting streptococci can be selected for use in developing lactic acid cultures. Some of the organisms that are normal in all respects are useless for this purpose; others combine satisfactorily with only certain *S. lactis* strains. *S. citrovorus* and *S. paracitrovorus* are equally useful. Good cultures have been obtained by employing both species in combination with *S. lactis,* although there is no advantage in this.

No correlation exists between the amount or rate of volatile acid production of a citric-acid-fermenting strain in milk and its suitability for developing cultures. Certain strains are especially useful and combine satisfactorily with various *S. lactis* strains. At the Iowa Agricultural Experiment Station a culture of *S. paracitrovorus* that was isolated from off-flavored sour cream combined satisfactorily with many *S. lactis* cultures. In general, a certain combination of strains is constant in its ability or inability to develop satisfactory lactic acid cultures. Organisms for use in the development of cultures can be obtained from various sources, such as spontaneously soured milk or cream.

Cultures developed from mixtures of organisms vary a great deal and the percentage that is entirely satisfactory is low. Apparently, it is difficult for the two types of organisms to establish a relationship that results in the formation of the products normally present in lactic acid cultures. Often the desired flavor does not appear, owing undoubtedly to failure of the flavor organisms to develop, and the culture suggests a pure culture of *S. lactis*. Some mixed cultures become satisfactory very quickly; others require a number of transfers. Unless there is an objectionable condition, or a complete lack of the desired flavor, a culture should not be considered a failure until it has been through at least 10 transfers in pasteurized milk. A culture that appears satisfactory should be carried for some time before a final decision is made with reference to it, since a stability that makes it possible to obtain good results regularly with a culture is very desirable. Use of the culture in the making of butter from high-quality cream provides valuable information, and when the keeping qualities of such butter are determined the test of the culture is complete.

SPECIAL CULTURES FOR BUTTER MANUFACTURE

The usual lactic acid cultures are employed in the butter industry with very beneficial results when a product with considerable flavor is desired. One of the difficulties with them is their variability, even under well-controlled conditions. This variation is especially serious when the culture needed for the day fails to coagulate or to develop the desired flavor.

Use of a pure culture of *S. citrovorus* or *S. paracitrovorus*. The possibility of using a pure culture of *S. citrovorus* or *S. paracitrovorus* for the development of flavor in butter has been studied by various investigators. Hammer[61] found that with added citric or lactic acid either *S. citrovorus* or *S. paracitrovorus* is capable of bringing about a fermentation in sweet cream that results in a desirable flavor in the butter. Maddock[62] reported that, although butter of exceptionally fine flavor can be produced with cultures of *S. paracitrovorus* under laboratory control, the uncertainties under practical conditions, owing to the slow growth, are too marked to warrant their general use.

A special type of culture in which *S. citrovorus* or *S. paracitrovorus* is grown in milk and the milk then acidified and again incubated has yielded relatively large amounts of acetylmethylcarbinol plus biacetyl.[15] The method of preparation is to inoculate either species into thoroughly pasteurized milk, allow it to grow at 21.1 C (70 F) for 15 to 24 hr, add 0.15 per cent citric acid and about 0.3 per cent sulfuric acid (in mixed aqueous solution) with vigorous agitation, and then continue the incubation at 21.1 C for 20 to 24 hr. Before addition of the acids there commonly is no acetylmethylcarbinol plus biacetyl present, but after the *p*H is lowered formation of these materials is rapid; following extensive production of the carbinol plus biacetyl there is a decrease unless the action of the organisms is controlled by relatively low temperatures. With this method of acidification a satisfactory *p*H for most cultures of *S. citrovorus* or *S. paracitrovorus* is from 3.9 to 4.3, although different strains vary in this respect. Addition of the acids causes a rapid coagulation of the milk, and there commonly is a contraction of the curd and separation of whey. With the procedure the final products are very uniform, provided suitable cultures are used and the *p*H properly adjusted, because the growth of only one species of organism is involved. At the Iowa Agricultural Experiment Station these cultures were found very useful for the production of flavor in butter. The period required for their development is a disadvantage and certain plants lack facilities for maintaining pure cultures of *S. citrovorus* or *S. paracitrovorus.*

In attempts to use pure cultures of *S. citrovorus* or *S. paracitrovorus* in the manufacture of various products, it should be recognized that under certain conditions their growth may be favored by the presence of *S. lactis.* Hansen [63] found that extracts of *S. lactis* stimulated development of *S. citrovorus* and also of *L. casei,* both the end point of fermentation and the final bacterial crop being increased; slight if any stimulation was noted when *S. lactis* was grown in media enriched by its own cell contents. Preparations of autolyzed cells or sonic vibrated cells were much more potent than non-treated control suspensions of killed cells.

Special species. Matuszewski and associates [64] isolated an organism which they designated *Streptococcus diacetilactis* that produces considerable amounts of *d* lactic acid and acetylmethylcarbinol in milk; the yield of carbinol is increased with added sodium citrate. They suggested this organism for use in the development of flavor in butter. Van Beynum and Pette [65] obtained an organism that rapidly produces *d* lactic acid in milk and vigorously ferments citric acid with production of acetic acid, carbon dioxide, acetylmethylcarbinol, and biacetyl; they designated it *Streptococcus citrophilus.*

SLOW LACTIC ACID CULTURES

Lactic acid cultures carried in laboratories or in plants sometimes develop acid slowly and may even fail to coagulate during an extended period. In some instances the change in rate of coagulation is gradual; in others it is very sudden and a culture that coagulates normally on one day is conspicuously slow the next. Delayed coagulation in cultures has been ascribed to various causes but bacteriophage seems to be of primary importance in this connection. Difficulties in dairy plants as a result of inhibition of cultures by phage appear to be increasing in a number of the important dairy countries. Phage is active against single strains of lactic acid organisms as well as against the usual lactic acid cultures.

Bacteriophage. In 1933, the Iowa Agricultural Experiment Station[66] reported that addition to pasteurized or sterilized milk of cultures that had coagulated slowly often caused considerable delay in coagulation of the milk by normal cultures; the restraining agent passed a bacteria-proof filter and was destroyed by boiling. Whitehead and Cox[67] isolated bacteriophage by the usual methods from an aerated culture which failed to develop acid. After propagation in serial culture for several weeks, it was obtained in such strength in a bacteria-free filtrate that one part of filtrate could be diluted with several hundred thousand million parts of milk and still lyse a susceptible organism. With the organism on a solid medium, the phage gave the classic appearance of plaques, lytic action spreading from the hypothetical phage corpuscles to form clear spaces in the mass of confluent growth. The phage appeared to be specific in its action on one strain of *Streptococcus.*

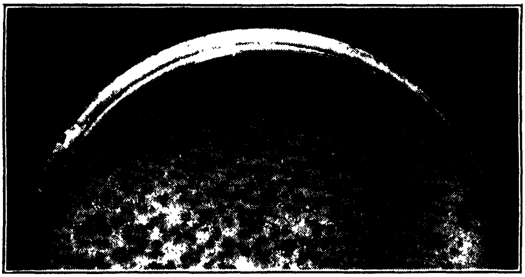


FIG. 31. Section of plate showing action of bacteriophage of *Streptococcus lactis.* Courtesy R. V. Hussong.

In the trials of Nelson *et al.*[68] retardation of acid production often resulted when a slow culture was added to a normal culture at the time of inoculation. Plate counts showed that slow acid development was due to slow multiplication of the culture organisms. When bacteria-free filtrates from cultures were added to freshly inoculated *S. lactis* cultures or regular lactic acid cultures, a majority of the filtrates from slow cultures and a smaller percentage from apparently normal cultures markedly restrained acid production and multiplication of organisms. Bacteria-free filtrates from slow and from normal cultures varied widely in their abilities to inhibit different strains of *S. lactis.* Almost every filtrate from a slow culture prevented normal acid development by at least one strain of *S. lactis* and most filtrates were active against several strains. Filtrates from normal cultures often contained inhibitory principle, but usually they were active against only one or two closely related organisms. It appeared that at least part of the difference between slow and normal cultures was that slow cultures contained enough strains of inhibitory principle to inhibit a substantial fraction of the *S. lactis* types present, whereas normal cultures contained either no inhibitory principle or only one or two strains of limited activity. The inhibitory principle was not affected by freezing and, at the proper *p*H, only slightly by drying. At approximately neutral reactions, some strains survived 70 C (158 F) for 10 min but not for 15 min; other strains were inactivated by less heat. The principle was more resistant to heat than its homologous organism. It was inactivated by crystal violet, methylene blue, hydrogen peroxide, and potassium permanganate. The strains studied were most active in neutral or slightly acid reactions.

Whitehead and Hunter [69] found with nine phage strains that thermal death points approximated 70 to 75 C (158 to 167 F) for 30 min at *p*H 6.0; optimum temperatures were between 30 and 37 C (86 and 98.6 F). The outstanding difference between them was their specific action on various organisms. The investigators reported that active chlorine is the most effective and convenient agent for destruction of phage. Hydrogen and hydroxyl ions inactivated phage when in sufficient concentration but their effects between *p*H 4 and *p*H 7 were negligible during several days at room temperature.

Phage strains active against lactic acid streptococci were found in the atmospheres of commercial cheese factories by Whitehead and Hunter.[70] Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for air-borne phage, but whey-contaminated dust also may play a part. The concentration of air-borne phage sometimes was so great that with normal technic it was impossible to prevent infection of the cultures for more than a few transfers. Protection from air-borne phage eliminated failures due to phage.

Sutton [71] noted that phage in milk cultures was not affected by 1 hr irradiation but when present in thin films or droplets of whey, or in whey dust, it was completely inactivated by irradiation. He suggested incidence of phage might be decreased by use of irradiation hoods. Wolf *et al.*[72] reported that fine mists containing 0.003 to 0.02 ppm chlorine usually gave satisfactory destruction of air-borne phage. With less than about 50 per cent relative humidity, hypochlorite solutions caused slight or negligible destruction of phage particles.

Multiplication of phage under suitable conditions must be extremely rapid to account for its effect in stopping the activity of certain cultures. Anderson and Meanwell [73] found that a minute infection of bulk culture milk with phage will increase a thousandfold during overnight incubation in the presence of susceptible culture and also that phage will retain its full strength on storage.

Hunter [74] noted that the presence in cheese milk of phage active against lactic acid organisms used to inoculate the milk leads to its multiplication during the cheesemaking process. The extent to which the phage is evident in the vat depends largely on the initial infection. A heavy infection may result in lysis of the organisms and consequent cessation of acid development, a light infection may have no noticeable effect, and an intermediate infection may affect the rate of acid development in the later stages of the process but within certain limits there is little adverse influence on the quality of the cheese.

Control of phage outbreaks. In some instances outbreaks of slow cultures, that evidently are due to phage, rather quickly disappear without any special control procedures. Frequently, however, the defect tends to persist, with variations in degree from day to day and perhaps from week to week, unless a resistant culture is obtained; it may become so serious that tanks of buttermilk, vats of cottage cheese, etc., are lost because of the great delay in acid development. One of the confusing things in an outbreak of phage is that there may be a distinct difference in the rates of acid development in two vats of milk set with the same lot of culture.

When several cultures from various sources are carried in a plant, one or more may prove resistant to the phage that is active against others and thus provide an immediate solution of the difficulty. If this is not the case, cultures should be obtained from a source other than the one supplying those causing the irregularity. In many instances a culture from a new source quickly establishes normal acid production in the plant operations. Presumably, the new cultures are resistant to the phage causing the defect or are free of serious phage contamination and remain in that condition. Numerous outbreaks of considerable duration have been immediately terminated by bringing to the plant a culture from a new source. On the other hand, with the new cultures coming from the same source as the old ones, outbreaks have continued, even with all possible precautions in carrying the cultures.

Termination of a phage outbreak by introduction of cultures from a new source is no guarantee for the future. Instances have been encountered in which the new cultures give normal acid production for an extended period and then fail. In some plants cultures from new sources are obtained rather frequently in an attempt to avoid irregularities in the culture operations. Those that are not needed immediately can be kept frozen for use in an emergency.

The opportunities for phage to get into the air undoubtedly are greater in certain types of plants than in others. Perhaps they are greatest in cheese plants with milk, curd, and whey to be handled, often with considerable agitation that results in fine moisture droplets being thrown into the air. Open whey separators are of special importance in this connection.

In order to minimize difficulties from slow cultures, all possible precautions should be taken in the normal handling of the cultures. Separate rooms for transferring are advisable to reduce the opportunities for entrance of phage from the air of the plant. Such rooms can be at some distance from the part of the plant in which cultures are used, or in a separate building. In some instances transferring the cultures away from the usual plant operations has avoided slow cultures when previously they occurred rather frequently.

Phage apparently can be carried on equipment and in various other ways and can be present in the milk coming from the farms. Some of these sources may be of importance under certain conditions. When chemical treatment of equipment, air, etc., seems advisable, chlorine sprays can be used.

An ideal solution of the phage problem would be some treatment which would destroy any phage present in a culture and leave the organisms in an active condition. Attempts along this line, using either chemicals or heat, have been unsuccessful. In general, the phage is more resistant than the homologous organisms. In this connection, Hunter[75] found that phage strains show a wider diversity of reaction to temperature conditions than the homologous organisms. They frequently have different optimum growth temperatures, quite distinct from those of the substrate organisms. Some strains failed to multiply at 37 C (98.6 F).

Milk from infected udders. Presumably, abnormal milk from infected udders sometimes delays the action of culture organisms but it does not appear to be involved in typical outbreaks of slow cultures. In such outbreaks attempts to detect an unusual condition in the milk commonly are unsuccessful. Moreover, practical experience indicates that with an active culture the rate of acid production is normal in many lots of milk that include considerable volumes from abnormal udders. In this connection, Davis [76] found that unheated blood serum may inhibit certain lactic acid bacteria, for example *S. lactis,* with a small inoculum of washed cells but that under conditions similar to those in practice no effect was noticeable so that it is unlikely slow cultures are ever attributable to infiltration of serum or its constituents.

Contaminating organisms. Various bacteria influence the rate of acid production by *S. lactis* so that contaminating bacteria would be expected to influence the rate of acid production by a lactic acid culture; presumably, some contaminants would accelerate and some delay acid production. Morgan and Curle [77] found that bacteria of the *B. subtilis* type sometimes caused slow development in lactic acid cultures.

"Non-acid milk" is a term used to designate milk which hinders acid development in a cheese vat by a normally active culture. Whitehead and Riddet [78] isolated, from certain milk supplies, a *Streptococcus* that during its growth in milk produced an inhibitory substance capable of delaying growth of normal lactic acid streptococci subsequently added to the milk. The substance was not destroyed by heating to 100 C (212 F) for 30 min. Streptococci which produced a substance inhibitory to lactic acid streptococci were detected rather frequently by Cox and Whitehead; [79] presence of the inhibitory substance was demonstrated by a special enrichment technic. Apparently, the organisms existed irregularly and in small number in the udders of some cows, in cow dung, in silage, and on the coats of animals. Hunter and Whitehead [80] noted that milk containing the inhibitory substance produced by the streptococci studied can cause delayed coagulation simulating that caused by phage.

LABORATORY EXAMINATION OF LACTIC ACID CULTURES

In the control of the lactic acid cultures used in a plant, daily examinations for flavor and general condition are essential if the proper quality is to be maintained. Frequently, such examination can be advantageously supplemented with various laboratory tests.

Microbiological examination. Cultures may contain contaminating organisms that are not evident from the flavor. Those that differ in morphology from the normal culture species can be detected by examination of stained preparations. Molds, yeasts, and certain bacteria, when present in relatively large numbers, readily are shown in this way. Cultures on agar also are useful in detecting contamination. They can be prepared in plates or on agar slopes, using standard agar or some other medium; acidified potato dextrose agar (*p*H 3.5) can be employed as a selective medium for molds and yeasts. Cultural methods permit examination of relatively large amounts of material and constitute a much more severe test than microscopic examinations.

Chemical examination. Under certain conditions it may be advisable to determine the acetylmethylcarbinol plus biacetyl in a culture, or the biacetyl can be determined separately. Various colorimetric procedures have been suggested for such determinations.[81]

The creatine test, for detecting acetylmethylcarbinol in bacterial cultures, has been adapted to the examination of lactic acid cultures and gives useful information on the general content of the carbinol plus biacetyl. It is carried out as follows: [82] 2.5 ml of the culture is poured into a test tube having an inside diameter of about 0.5 in. and a small amount of creatine (a portion of the powder equal to about one-third of the volume of a wheat grain) added; the creatine can be handled best on the point of a small knife. Strong sodium hydroxide (40 gm made up to 100 ml with water) equal in volume to the volume of culture then is poured into the tube, the mixture thoroughly shaken, and allowed to stand. If relatively large amounts of acetylmethylcarbinol plus biacetyl are present in the culture, a red color develops at the surface of the mixture in a few minutes or less, and soon extends to a depth of 0.25 in. or more. With smaller amounts of acetylmethylcarbinol plus biacetyl the red color develops more slowly and the colored portion is comparatively shallow. Cultures that are decidedly lacking in flavor may show no red color even after holding the test mixture for hours. When a strong reaction is obtained, the material adhering to the wall of the tube above the surface of the liquid is strikingly red in color. Formation of only a thin red band on the surface of the test mixture should not be interpreted as indicating that the flavor of the culture is entirely satisfactory, since this may occur with cultures which contain relatively small amounts of acetylmethylcarbinol plus biacetyl. A culture with a high flavor rapidly gives a rather broad band of red color.

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Chapter 15

Bacteriology of Fermented Milk Preparations

Consumption of milk that has undergone extensive changes as a result of growth of micro-organisms is common in all the countries in which milk is readily available. The products have a high food value and, in addition, some of them appear to have therapeutic properties in certain conditions, especially intestinal disorders. Their use is increasing in many parts of the United States and in some localities the fermented milk sold makes up a considerable percentage of the total milk sales.

There are two main types of fermentations used with fermented milk, an acid fermentation and a combined acid and gassy fermentation, although contaminating organisms often are present and may influence the changes that take place. The former occurs in various types of products and the latter in such products as kefir and kumiss.

Buttermilk. Buttermilk, in the strict sense, is the material remaining when the fat is gathered in the process of buttermaking. If the cream that is churned has undergone a clean, acid fermentation the buttermilk has a flavor that is pleasing to many people. Churning of butter on farms from naturally soured cream that has been properly held frequently yields a fine-flavored buttermilk, and the same is true when high-quality sweet cream is delivered to creameries, pasteurized, and lactic acid culture added. If the cream is old and has undergone a variety of fermentations, the flavor of the buttermilk often is unsatisfactory. This is especially true when the cream has been treated with neutralizer. Pasteurization of cream in a sour condition usually causes the casein to harden in particles, so that, when the buttermilk obtained is allowed to stand, these quickly settle, leaving a more or less clear whey-like material at the surface.

Because of the difficulty of obtaining buttermilk of high quality from the cream delivered to many creameries, various products are made from skim milk and/or whole milk and sold as culture or artificial buttermilk, or under some special name adopted by the manufacturer. In the preparation of these materials the fermentation commonly is carried out with the cultures used in the butter industry so that the product is essentially lactic acid culture. The acidity developed should not be too high, preferably about 0.75 to 0.85 per cent, although some persons prefer a very acid product. After the fermentation, the product is thoroughly agitated in a vat or churn to give it a smooth, creamy texture. Whether the buttermilk is to be made from skim, part skim, or whole milk depends on the price for which it is to sell. For many consumers the presence of fat makes the product more pleasing. When the fermentation is carried out in whole milk the fat often forms firm masses at the surface that cannot be broken easily and so a product containing fat sometimes is prepared by fermenting skim milk and adding cream after the fermentation is complete. Occasionally, butter granules are added to culture buttermilk to give it an attractive appearance, or cream is added and granules produced by churning.

The importance of citric acid as a source of flavor constituents in lactic acid culture has led to its use in making culture buttermilk. Nelson [1] reported that addition of 0.15 per cent citric acid crystals, dissolved in distilled water, to pasteurized skim milk to be made into culture buttermilk regularly gave the finished product an improved flavor. The creatine test showed that the added citric acid resulted in more acetylmethylcarbinol plus biacetyl in the product.

The strain of lactic acid culture used influences the consistency of culture buttermilk. The normal consistency can be reduced by adding pasteurized sweet skim or whole milk and thoroughly agitating. A satisfactory ratio is 1 part of skim or whole milk to 2 parts of fermented milk, but this can be varied widely.

Salt sometimes is added to buttermilk to bring out the flavor. Buttermilk occasionally is modified by addition of various materials, such as sugar, lemon extract, lemon juice, and beaten egg, the amounts being varied to suit the taste; other fruit juices or extracts can be employed in place of lemon.

Bulgarian milk. In some instances a culture milk containing *L. bulgaricus* is desired for special purposes. Cultures of *L. bulgaricus* are much more difficult to propagate in pasteurized milk than lactic acid cultures because they must be grown at 32.2 to 43.3 C (90 to 110 F) and these temperatures are very favorable for the growth of organisms resisting the pasteurization exposures. Pasteurization on 2 successive days at 82.2 to 93.3 C (180 to 200 F) for 30 min commonly aids in avoiding undesirable fermentations, as does the selection of milk with a low bacterial count. Sterilized milk can be employed and is very satisfactory except for the heated flavor and the color produced by the high exposures necessary. Cultures of *L. bulgaricus* used for inoculating large volumes of milk should be carried in sterilized milk to ensure inoculation of pure cultures. Milk fermented by *L. bulgaricus* does not have a flavor that is pleasing to most persons and the flavor can be improved by adding lactic acid culture to it. Attempts made to grow *L. bulgaricus* and the culture organisms together usually have not been successful because of the difficulty of maintaining a satisfactory balance between the various species.

Cultures of *L. bulgaricus* have been employed to prevent wheying off of buttermilk [2] obtained when sour cream is pasteurized and churned. A ropy strain of *L. bulgaricus* is used and milk fermented by it is added to the buttermilk and thoroughly distributed. The heavy body of the milk fermented by the ropy *L. bulgaricus* tends to prevent settling of the casein in the buttermilk. The amount necessary is variable, depending on the condition of the buttermilk, the period it is to stand, etc., and should be determined by trial. Addition of lactic acid culture also has an effect in preventing wheying off of buttermilk and its use improves the flavor.

Bulgarian milk has not given satisfactory results in many of the attempts made to control the intestinal flora through the consumption of milk cultures of it, and milk fermented by *L. acidophilus,* a normal intestinal form, is more widely used.

Acidophilus milk. Milk fermented by *L. acidophilus* is marketed under the name of acidophilus milk.[3,4] This organism also is carried in pasteurized milk with considerable difficulty; it grows comparatively slowly and the temperatures used, which ordinarily are from 35 to 37.8 C (95 to 100 F), are very favorable for the species resisting pasteurization. *L. acidophilus* is most commonly grown in sterile milk, either in flasks or in special tanks that permit heating the milk under pressure. Controlled methods of producing the original milk make it possible to use low sterilization exposures and thus to obtain a product with less heated flavor and less change in color. Cultures used for inoculation must be carried under controlled conditions to ensure purity, since a few foreign organisms may quickly dominate the fermentation. Micrococci are common contaminating species, although spore formers also are encountered; the micrococci presumably are the result of contamination from the air. *L. acidophilus* sometimes is grown in whey instead of milk.

In general, it appears that large doses of *L. acidophilus* organisms taken regularly over a period of days or weeks, especially with milk or certain carbohydrates, tend to establish *L. acidophilus* as a predominant intestinal organism, frequently to the exclusion of certain other types. Lactose appears to be a particularly satisfactory carbohydrate, presumably because of its relatively slow absorption from the intestinal tract; for consumption it can be dissolved in water or milk.

Results of various investigations indicate that *L. acidophilus* colonizes in the intestinal tract much more readily than *L. bulgaricus.* A number of theories have been proposed to explain this difference between the two species but none of them adequately shows why *L. acidophilus* develops well in the intestinal tract and *L. bulgaricus* does not. Some strains of *L. acidophilus* apparently colonize more successfully than others.

In the use of acidophilus milk, viability of the organisms is of primary importance. Apparently, the holding temperatures should not be too low. Kulp [5] found that in order to ensure satisfactory viability for 2 days to 1 week commercial acidophilus milk of good quality may be stored at 5 C (41 F), provided the acidity at bottling is not above 0.65 per cent. When a satisfactory viability for more than 1 week is desired, the following points are important: (1) Numbers of foreign bacteria must be negligible; (2) there must be rigid precautions against excessive acidity; (3) storage temperature should range between 12 and 16 C (53.6 and 60.8 F), the optimum viability temperature for *L. acidophilus* in milk being 16 C with an initial acidity of 0.65 per cent.

Kopeloff *et al.*[6] reported that, in milk at the same titrable acidity, viability of rough and smooth strains of *L. acidophilus* differed at 4 C (39.2 F) as well as 20 C (68 F). The rough strain lost its viability rapidly at 4 to 9 C (39.2 to 48.2 F), as compared with storage at 20 or 25 to 30 C (68 or 77 to 86 F). With an initial titrable acidity of 0.6 to 1.0 per cent, the original count diminished approximately 90 per cent in 3 days in the ice box and between 20 and 75 per cent at room temperature. The smooth strain lost its viability less rapidly than the rough strain but storage in the ice box was more detrimental than storage at room temperature.

For persons who dislike the flavor of ordinary acidophilus milk Myers [7] suggested the use of unfermented acidophilus milk, which consists of highly pasteurized sweet milk with many *L. acidophilus* cells suspended in it. The organism is grown in a sterilized medium and the cells then separated and added to the milk. Unfermented acidophilus milk is handled in the same manner as market milk and when stored at 2 to 5 C (35.6 to 41 F) keeps sweet as long as 7 days. Souring is rapid at room temperature, 10 C (50 F) being the maximum temperature at which the milk can be stored satisfactorily. With the product, successful implantation of *L. acidophilus* in the intestinal tract was obtained in rats and humans.

L. acidophilus also has been included in sherbets, candies, jellies, and various other materials; maintenance of viability of the organisms is a problem with all of these products. Prouty and Bendixen[8] found it possible to prepare sherbets in which large numbers of *L. acidophilus* remained viable after 5 to 7 days at temperatures below −17 C (1.4 F), when the more resistant strains with a high initial count were used and the titrable acidity was not permitted to become excessive, preferably not above 1 per cent.

Kefir. Kefir[9] is a type of fermented milk in which there is development of both acid and gas. It apparently originated among the people living in the Caucasus Mountains and surrounding territory and is made from milk of various species of animals. The fermentation can be started through use of kefir grains, which are convoluted masses made up of milk constituents and many micro-organisms. The grains are transferred from fermented milk to lots of fresh milk or they can be dried and preserved and then soaked when needed. In the dry condition they are yellow; during active growth they are almost white. When added to milk the grains settle to the bottom but in the fermentation process they may be carried to the top by the gas developed. The grains increase in size during continued use and occasionally break up so that an abundant supply is provided. Under primitive conditions the fermentation commonly is carried out in leather bags and, as the kefir is used, fresh milk is added. The bags are kept outside during the summer, in either the sun or shade depending on the temperature, but in winter they are kept in the house. When kefir is to be removed the portion wanted frequently is tied off from the remainder so as to prevent escape of large quantities of gas when the bag is opened.

Fig. 32. Actively growing kefir grain.

Various organisms have been isolated from kefir and it is probable that the species present are not constant. Streptococci and lactobacilli are involved in the acid development and the gas often is produced by lactose-fermenting yeasts. Alcohol in variable quantities is formed along with the gas; it is small in amount in the freshly made product but may increase considerably with age.

A product showing an acid and gassy fermentation can be made without kefir grains. The acid fermentation commonly is carried out first and gas production then is brought about through action of a yeast; if sealed containers are employed, the carbon dioxide can be largely retained. The general procedure is as follows:[10] Milk is fermented with a lactic acid culture in the usual way. It is put into bottles, about 4 per cent cane sugar added and dissolved, and then a small amount of bakers' or brewers' yeast, suspended in water, is inoculated. The bottles are tightly stoppered, the corks wired in if they are not of the picnic type, and the material held at about 21.1 C (70 F). Cane sugar is added because the yeast does not ferment lactose and for many persons sugar improves the flavor of the product. When the fermentation is sufficiently advanced the milk should be cooled to check growth of the organisms. Cooled bottles also can be handled with less danger of breakage than uncooled ones because of the greater solubility of the gas at lower temperatures. It is not unusual for a bottle to be shattered by the gas pressure. A product showing acid and gas can be made from whey[10] by the general procedure outlined. There is less danger of bottle breakage with whey than with milk. With milk the casein frequently is in masses, whereas with whey casein is present only in negligible amounts.

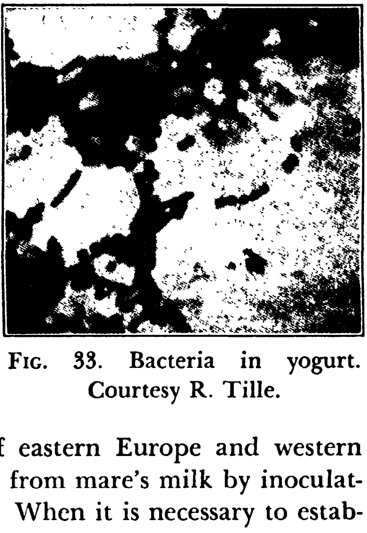

FIG. 33. Bacteria in yogurt. Courtesy R. Tille.

Kumiss. Kumiss, which is a product showing an acid and gassy fermentation, is made in certain parts of eastern Europe and western and central Asia. It commonly is made from mare's milk by inoculating with milk that has been fermented. When it is necessary to establish the fermentation, some fermenting or decaying material, such as flesh, tendon, or vegetable matter, can be added to the milk. The flora of kumiss apparently varies just as does that of kefir. Milk of the mare is of somewhat different composition than that of the cow, usually containing more water and sugar but less fat, protein, and ash.

Yogurt, mazun, etc. In certain parts of the world milk from various animals is fermented by inoculation of milk containing acid-producing organisms. The product is called yogurt, mazun, gioddu, dadhi, leben, etc., depending on the country in which it is made. The milk commonly is boiled before it is inoculated and the boiling may be continued until the milk is greatly concentrated. Under more modern conditions, concentrated or dry milk solids may be added to milk to raise the solids content and the mixture homogenized and pasteurized before inoculation. Incubation usually is at 40 to 45 C (104 to 113 F) and coagulation is very rapid. Because of the high solids content, the product is very thick and often is eaten with addition of various materials, such as sugar, bread, and dates. The acid-producing organisms usually are lactobacilli and streptococci. In some instances organisms are found that play no part in the acid development. Rosell[11] noted that in the preparation of yogurt 3 species of bacteria are desirable.

Holding fermented milk. One of the serious problems with fermented milk is keeping it in a good condition after the fermentation is complete. With the large numbers of organisms present, growth is likely to continue after the desired stage of development is reached and the product then deteriorates. With buttermilk, low temperatures provide the most satisfactory method of holding and, if the product is to be kept for some time, it should be held at 4.4 C (40 F), or lower. The quality of buttermilk offered for sale often is poor; frequently, the product served has a yeasty and moldy flavor and is quite unsatisfactory to anyone familiar with a high-quality product. While the acid developed in buttermilk may prevent growth of many organisms, certain species can tolerate it, the yeasts and molds being especially important. Manufacture and handling of buttermilk under such conditions that yeasts and molds are not present is an aid in marketing a high-quality product but the real solution of the holding of buttermilk is employment of storage temperatures that are so low growth of all organisms is materially delayed.

With acidophilus milk, holding temperatures should be used that favor retention of viability by *L. acidophilus.* When a fermented milk involves formation of gas as well as acid, holding under unsatisfactory conditions may result in bursting of the containers; it is necessary to keep the containers closed to retain the gas that is so important in giving the product the desired flavor.

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Chapter 16

Bacteriology of Butter

The bacteriology of butter involves considerations that differ essentially from some of those important in the bacteriology of milk. Lactic acid culture sometimes is added to pasteurized cream intended for butter, or the cream is actually ripened with culture, and many of the culture organisms are carried into the finished product so that the initial bacterial content of butter may be high, owing to organisms intentionally included. Because of its composition, butter is less satisfactory than milk for growth of various micro-organisms. Lactose, which is readily attacked by many species, is present in small amounts, whereas approximately 80 per cent of the product consists of the relatively resistant fat. The moisture content is low and, except with unsalted butter, contains a comparatively high concentration of sodium chloride. Moreover, the moisture is in droplets that are surrounded by fat so that water is not the continuous phase as it is in milk. Most of the organisms are in the water and, since there are many more droplets than there are organisms, a large proportion of the droplets are sterile and must remain so because bacteria cannot migrate through the fat. The holding period with butter often is comparatively long because of the time required for butter to pass through the usual wholesale and retail channels to the consumer. Butter in storage is held at −17.8 C (0 F), or lower; in stores and homes the temperatures usually are such that many bacteria can multiply, provided they tolerate the other conditions imposed by the butter.

From the standpoint of the microbiology of butter there is a striking difference between the salted and the unsalted products. The salt definitely inhibits growth of various species of organisms and may even kill some of them so that deterioration through growth of organisms presents more of a problem with unsalted than with salted butter. On the other hand, certain undesirable chemical changes, favored by the salt, occur more readily in the salted product.

Determination of numbers of bacteria. The numbers of bacteria in butter can be determined by the plate method or the microscopic method, or by an adaptation of the Burri technic. All commercial butter is made from pasteurized cream and many of the bacteria in stained preparations from butter are dead so the microscopic picture must be interpreted properly. Direct observations on the organisms in defective butter often supply information that is useful in establishing the cause of the abnormality.

Because of its physical condition, the sampling of lots of butter presents difficulties not encountered with milk. Standard methods[1] of sampling butter in churns, in tubs or packages, and in prints, and for the care of samples have been developed.

Plate count. Plating of butter is more difficult than the plating of milk because of the solidification at ordinary temperatures, and the tendency of the fat to form large globules in the medium even when it is melted. Water blanks, pipettes, and plates should be warmed to a temperature above the melting point of the butter; this can be done conveniently by allowing them to stand in a 45 C (113 F) incubator, or the blanks can be placed in warm water. The portions for plating can be weighed, using precautions against contamination, but it is more convenient to place the sample jar in a water bath at 40 to 45 C (104 to 113 F) until the butter can be shaken to a thin, creamy consistency, and then to obtain the portions required with a warmed pipette. The outside of the pipette is kept relatively free of butter and the pipette is emptied as completely as possible; when butter is being added to a water blank the pipette is rinsed by drawing the warm water into it. After the butter is in the first water blank, dilutions are made as with milk. Because the fat quickly comes to the top of a blank on standing, it is necessary to work rapidly and to shake the blank just before a portion is removed. Excessive shaking, especially if the butter is not thoroughly melted, may cause the fat to form clumps and thus interfere with its distribution. The dilutions used depend on the number of organisms expected from the history of the sample.

Standard, beef infusion, and various other agars can be used for the plating. White[2] found that in plating sweet cream butter made without culture beef infusion agar containing 1 per cent lactose gave a better development of bacterial colonies than various other media. By addition of sterile milk and/or emulsified fat to a medium, differential counts can be made. Tomato and whey agars are useful in plating butter containing lactic acid culture organisms. Incubation of the plates 3 to 5 days at 21.1 to 25 C (70 to 77 F) is advisable; in some cases an additional incubation of 2 days at 37 C (98.6 F) is used. Butter that has been held for some time at temperatures approximating 0 C (32 F) may contain many organisms that are so definitely psychrophilic they fail to grow at 37 C.

Microscopic count. Various procedures have been used in making microscopic counts on butter. Hammer and Nelson[3] developed a method in which 10 ml of butter that has been warmed to 45 C (113 F) is centrifuged in a separatory funnel, and the serum drawn off and examined essentially as in the microscopic count on milk. A 0.01-ml portion of the thoroughly mixed serum is spread over an area of 1 to 8 or more sq cm, depending on the number of organisms expected; if the area is larger than 1 sq cm distilled water, or milk free of stainable cells, is used in the spreading. Results are calculated to the basis of 1 ml of serum, and then to the basis of 1 ml of butter by dividing by 9, since there are only insignificant percentages of the organisms left in the fat and the volume of the serum is approximately one-ninth of that of the original butter.

Fay[4] suggested a method for the microscopic examination of butter in which the butter is melted at 45 C and thoroughly agitated to ensure a homogeneous sample, after which 0.1 ml of the butter, 1 drop of xylene, and 1 drop of Mayer's egg-glycerol mixture * are placed on a slide 1 by 3 in.; these are thoroughly mixed and spread over the entire slide with an L-shaped needle. The slide is placed on a flat bottle containing water at about 80 C (176 F) for 10 to 15 min to dry and to coagulate the albumin, after which it is fixed in 70 per cent alcohol for 10 to 20 sec and again dried. Following treatment with xylene it is stained with methylene blue and examined. With a field diameter of 0.157 mm, the number of bacteria per field is multiplied by 1,000,000 to obtain the number per milliliter of butter.

In the examination of defective butter general observations on the numbers and morphologic types of organisms present often are advisable. Hussong[5] suggested a procedure that can be applied quickly with very little equipment. A small piece of butter is placed on a slide and melted. A few drops of acetone are added and mixed with the butter by moving the slide or stirring with a sterile needle. The acetone-fat-water mixture is poured off gently, leaving the curd and other materials on the slide. A second treatment with acetone completes removal of the fat. After evaporating the remaining acetone, the slide is stained and examined.

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* Mayer's egg-glycerol mixture is prepared by mixing and beating equal parts of egg albumin and glycerol, filtering through cotton, and adding 1 per cent sodium salicylate as a preservative.

Burri technic. Long and Hammer[6] adapted the Burri smear culture technic to the examination of butter. The butter is placed in a sterile petri dish and brought to about 21 C (69.8 F) at which temperature small amounts can be picked easily. Under a binocular giving about a 6X magnification, small amounts—approximately 1/20,000 gm each is satisfactory—are picked with a flamed platinum needle; the binocular aids in keeping the portions uniform in size and in actually picking each portion rather than scraping it from a relatively large area. Each portion is spread on the surface of a dry agar slope; standard, beef infusion, tomato agars, etc., are satisfactory, depending on various factors. Ordinarily, 25 portions are picked but larger or smaller numbers may be used to give more or less detail in the results. The slopes are incubated at 21 C for 4 or 5 days and then counted. The amount of butter actually being picked can be estimated by determining the collective weight of a number of portions. The method gives results lower than the plate method, presumably because clumps of bacteria are not broken as they would be in a water blank; also, there may be overcrowding on slopes with high-count samples. The main advantage of the procedure is in studying the distribution of organisms from point to point in a sample.

Determination of numbers of yeasts and molds. The numbers of yeasts and molds in butter frequently are determined to obtain information on the efficiency of plant operations from the sanitary standpoint. With satisfactory plant methods, yeast and mold counts are very low; with unsatisfactory methods they often are high. Usually, yeast and mold counts are made with the plate method, but a microscopic colony count also has been employed. The organisms can be counted by examining stained preparations made from the butter serum; such values include both living and dead organisms.

Plate count. The numbers of yeasts and molds in butter are determined by plating on potato dextrose agar acidified to *p*H 3.5 ± 0.1 with tartaric acid to prevent development of bacterial colonies; the medium is convenient, inexpensive, and very satisfactory.[1] When the yeast and mold counts are low, plates must be poured with 1-ml quantities of butter. This requires properly warmed equipment and rapid manipulation, if the fat is to be well distributed through the agar; without such distribution some yeasts and molds may be completely enclosed in fat and their growth delayed or entirely prevented.

At one time whey agar, acidified by adding 1 ml of a sterile 1 per cent solution of tartaric acid crystals to each plate at the time of pouring, was widely used. Lund[7] employed wort agar to which 4 ml of a sterile 5 per cent lactic acid solution per 100 ml of medium was added just before plating. Stiritz[8] suggested agar made from malt beverage and added lactic acid in the proportion employed by Lund. Various other media also have been advocated. Hood and White[9] noted the variation in the *p*H of the different media proposed for yeast and mold counts and pointed out that some of them permitted growth of bacteria. They found that all bacterial growth was inhibited by a *p*H of 3.5. Wort, malt, whey, and potato were equally suitable as a nutrient base for yeast and mold counts when the *p*H was 3.5. Later, White and Hood[10] reported that no bacterial colonies were present on plates poured for yeast and mold counts at *p*H values of 3.4 and 3.8, but that with the majority of butter samples many were present at *p*H values of 4.2 and 4.6. Since *p*H values of 3.4 and 3.8 did not significantly affect the yeast and mold counts on butter, they considered a *p*H of 3.5±, which allows a margin of safety in standardizing the medium, the most satisfactory for routine analyses of butter for yeasts and molds.

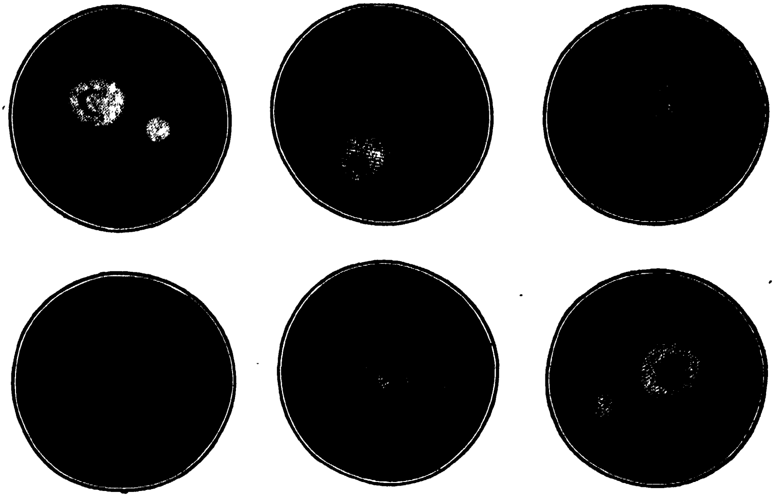

FIG. 34. Various types of yeast colonies on plates poured for yeast and mold counts on butter.

Clerkin[11] also noted that at a *p*H of 4.5 a large number of colonies that were included in the yeast counts were bacteria, and that the *p*H of the medium had to be reduced to about 3.5 before the counts could be regarded as consisting entirely of yeasts. The *p*H did not appear to have any marked effect on the mold count over the range investigated.

Various incubation conditions have been employed for the plates used in determining yeasts and molds in butter. In the studies of White and Hood [12] the average yeast and mold count of all samples, and counts for the majority of the individual samples, were slightly higher at 25 C (77 F) than at 30 C (86 F) with an incubation of either 48 hr or 5 days, although the differences were not considered of practical significance. At 37 C (98.6 F) the counts nearly always were appreciably lower than at 25 or 30 C. These results are in agreement with the general information on the growth temperatures of yeasts and molds, and plates now commonly are incubated 5 days at 21 or 25 C (69.8 or 77 F). If many molds that tend to overgrow the plates are present, it may be necessary to count the plates earlier.

Microscopic colony count. Johns [13] adapted the microscopic colony count to the determination of yeasts and molds in butter. An incubation of 12 to 18 hr at 25 C (77 F) was the most suitable; with a shorter period it appeared advisable to raise the temperature to 30 C (86 F), whereas with a longer period a lower temperature was desirable. Study of the method led to the conclusion that it furnished a reasonably accurate indication of the mold content of butter when this was above 100 per milliliter, as determined by the usual plate method; on the average the mold counts obtained were twice as high as those obtained with the usual method. Yeast counts showed greater variations from the values obtained with the usual method than did mold counts, but a satisfactory relationship between the results of the two methods was secured with 74.6 per cent of the samples.

Microscopic count. Redfield [14] proposed a method of counting yeasts and oidia in butter with an adaptation of the microscopic method for counting bacteria in milk. The butter is melted at 45 C (113 F) and allowed to stand at this temperature, or slightly lower, until the fat separates. The water and materials other than fat are mixed by filling and emptying a pipette, after which 0.01 ml is spread on a glass slide, care being taken to exclude the fat; the area covered is 1, 2, or 4 sq cm, depending on the number of yeasts and oidia expected and, if needed to obtain uniform smears, distilled water is used for dilution. The slide is dried, stained, and examined in the usual way. The method gives the count per milliliter of the mixture of moisture, curd, etc., and includes both living and dead organisms. It is limited in its usefulness because with many lots of butter the yeasts and molds are present in such small numbers they are not readily counted microscopically.

Mold hyphae in cream and butter. Under certain conditions molds grow extensively on cream and considerable numbers of mold hyphae, or filaments, may be present in cream as it reaches butter plants, and also in the butter made from it. The relative abundance of mold hyphae in butter has been employed in attempts to detect the use of unfit cream in butter manufacture and, accordingly, the presence of the hyphae in cream and butter has been studied extensively.

Molds in cream. Following preliminary work by Wildman,[15] a method for the visual demonstration of mold in cream was devised by Parsons.[16] It consists of flocculating the mold hyphae in 9 ml of cream with heat and a special reagent and filtering them on an organdy disc. The preparation thus obtained is compared with standards and classified. The method is rapid and gives results that are very satisfactory for determining the relative abundance of mold in different lots of cream. Some bacteria and yeasts commonly are flocculated with the mold hyphae but these probably have no great influence in the classification of the preparations.

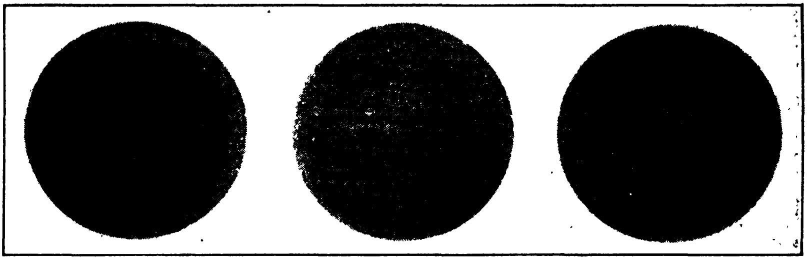

FIG. 35. Mold at different levels in undisturbed cream (Parsons visual mold test). *Left:* top 0.5 in.; *middle:* second 0.5 in.; *right:* second 1 in.

Sweet cream contains very few mold hyphae because, on cream held under such conditions that it remains sweet, growth of molds is negligible. Sour cream may contain few, moderate numbers of, or many mold hyphae, depending very largely on the conditions of holding. As ordinarily produced, cream contains molds and, if it is held under suitable conditions, growth will occur.

Various species of molds grow on cream but, in general, *O. lactis* is much more important in this connection than any other. The colonies or film, and later the wrinkled membrane, produced when it grows extensively on cream are very conspicuous and characteristic.

If the conditions of holding permit rapid development of organisms, other types of surface growth sometimes occur; these may be due to molds, yeasts, or bacteria, extensive growth of certain species of bacteria being made possible by the action of molds in increasing the *p*H of the sour cream.

Mold growth on unagitated cream is primarily at the surface and the top 0.5 in. of a lot of cream contains most of the mold, with the lower layers showing very little. Because of this, the relationship between surface area and volume of a lot of cream [17] is an important factor in determining its mold content. Cream in a shallow layer may contain a relatively large amount of mold when mixed and tested, whereas an equal volume of cream from the same original lot but held in a much deeper layer may show comparatively little because of the relatively small surface area.

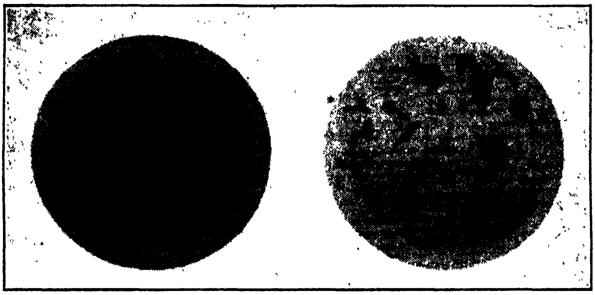

FIG. 36. Effect of surface area on mold in cream held 3 days at 21.1 C (Parsons visual mold test). *Left:* cream in shallow layer; *right:* cream with one-eleventh surface area.

Temperature and period of holding are important factors in determining mold growth on cream. Growth is rapid over a rather wide temperature range. There may be a variation from one lot of cream to another because of differences in the species or strains of molds present, but ordinarily growth is active from 21.1 C (70 F), or below, to 29.4 C (85 F), or above.[18,19,20] With some lots of cream growth is quite rapid at 15.6 C (60 F) but commonly it is very slow at 10 C (50 F), or lower. Above 35 C (95 F) growth often is slow, if it occurs at all, because such temperatures definitely are too high for most species.[19,20] At all temperatures permitting mold growth, it continues over an extended period and at favorable temperatures the mass of mold on the surface of the cream rapidly becomes very conspicuous.

Morrison and Hill[21] followed, microscopically, the growth of *O. lactis* on standard agar. At room temperature the average period for 10 colonies (each from a single spore) to produce 100 microns of mycelia was 10.7 hr, 500 microns was 14.2 hr, and 1,000 microns was 15.3 hr; at 14.4 C (58 F) the corresponding values were 44.3, 59.0, and 65.7 hr.

The extent of the contamination with molds obviously is a factor in their growth on cream but the great variations in growth rates under different holding conditions easily outweigh variations in the extent of the original contamination. Mold growth on heavily contaminated cream is controlled readily by low holding temperatures, whereas it can be very extensive on lightly contaminated cream held at favorable temperatures.

Relatively large shipments of cream tend to have lower mold contents than relatively small shipments;[20,22] various factors may influence this, such as surface-volume relationships, care in production, age, etc. High-fat cream tends to have less mold than low-fat cream.[17,20,23] Excessive acidities limit mold growth.[19,22] Rancid cream tends to be relatively low in mold,[20] presumably because of the mold-inhibiting properties of fatty acids. The humidity under which cream is held apparently does not affect mold growth on it;[20] however, under humid conditions contamination of cream with molds may be excessive because of the slow drying of utensils and perhaps other factors.

Elliker and Brown[24] found that, when cream was stored at 15.6 C (60 F) or 26.7 C (80 F) for 4 or 7 days, centrifugal separation resulted in lower mold contents than did water-dilution or shallow-pan separation. With fresh milk containing a comparatively large number of molds, centrifugal separation removed approximately 90 per cent from both the cream and skim milk. Water-dilution and shallow-pan separation tended to concentrate molds in the cream.

Various schemes of stirring or layering have been suggested to keep down mold growth on cream. The results obtained in different studies are not in agreement,[17,20,22,25] presumably because of variations in the procedures followed. Frequent stirring, for example, twice a day, tends to keep down mold growth, but does not control it; stirring once a day may have little effect or may tend to increase it. Attempts to add new portions of cream to an accumulated lot by pouring the new portions so that they will float on the accumulated lot ordinarily have not kept down the mold content of cream; often there is a tendency for the mold growth to float to the new surface. Flooding the surface of cream with such material as mineral oil[17] largely controls growth of molds there by limiting the air supply, but such a procedure has no practical application.

Molds in butter. Wildman [15] determined the relative abundance of mold hyphae in different lots of butter. The method involves microscopic examination of the product under specified conditions,[1] with the reporting of the results on the basis of the percentage of positive fields. Adequate attention to all the details of the procedure is essential.

Elliker [26] developed a method for determining the combined length of mold fragments per milligram of butter. It involves diluting 1 gm of butter with hot gum solution, spreading a portion of this on a glass slide, staining, and examining with low-power magnification. The average length of mold filaments per field is determined and the results calculated in millimeters per milligram of butter.

Ordinarily, the mold hyphae seen in examining butter by either of the methods are dead as a result of pasteurization of the cream, which is the primary source of the hyphae in butter; accordingly, butter containing much mold may show negligible numbers of colonies on culture. Butter with a high hyphae content normally comes from cream containing a large amount of mold growth, particularly of *O. lactis.* Poorly cleaned equipment, etc., are other possible sources of mold hyphae in butter.

The Parsons [16] procedure for visual demonstration of mold in cream can be applied to butter and comparisons of mold contents of different lots of butter made very satisfactorily. With it, examinations are made rapidly, compared to any counting or measuring procedure; by developing a set of standards, the results can be rather closely related to the results of other procedures for estimating mold contents of butter.

Butter made from sweet cream regularly has a very low hyphae content. Butter made from sour cream may have a low, moderate, or high content, depending largely on the conditions under which the cream is held, although extent of the contamination also is a factor.

Retention in butter of hyphae in cream. Adams and Parfitt [17] found that, of the molds in cream, less than 10 per cent to as high as 70 per cent were retained in the butter and that in 66.2 per cent of 121 comparisons the retention was below 31 per cent. Size of the butter granules and degree of neutralization of the cream did not affect the mold retention. Elliker [27] reported that butter usually retained more than 50 per cent of the total length of mold filaments in cream and that the wash water contained a very small percentage. During churning the butter appeared to retain the long filaments, whereas most of the tiny fragments passed into the buttermilk.

Comparison of flavor grade and mold content of butter. Rapid growth of molds in cream commonly is accompanied by growth of other organisms and by flavor deterioration so there is a general relationship between the mold content of cream and its organoleptic quality. However, it is only general because factors other than mold growth are involved in flavor deterioration of cream. As a result, some butter scoring relatively high for a sour cream product may have high hyphae contents and some butter scoring very low may have low hyphae contents.

Various investigators have noted the lack of correlation between flavor grade and mold content of butter. For example, Elliker and Horrall[28] studied 1,385 samples of commercial butter and found that the mold mycelia values did not correlate closely with organoleptic grades over the various seasons. The values showed the expected pronounced seasonal variation, with higher mold contents in the warm months. Organoleptic grades appeared fairly uniform throughout the seasons, although slightly more poor-quality butter appeared to be manufactured in the fall. Results for all seasons indicated that, by mold mycelia standards, over 50 per cent of all cooking-grade butter, almost 75 per cent of 89 score, about 90 per cent of 90 score, and all of 92 score butter, was legal. The authors considered that, in actual quality of butter in the area studied, greater improvement might be made if more attention was paid to organoleptic grade of cream and less to mold content.

Certain areas apparently produce butter with a generally higher hyphae count than other areas supplying butter of the same general grade. Some butter organizations operating over a large area have found the mold hyphae problem regional. For the most part, northern states, with shorter summers and cooler well water, have lower hyphae counts than southern states, even with butter of essentially the same grade. Again, butter from the southeastern states seems to be higher in hyphae than butter of comparable grade from the southwestern states. In certain areas, winter butter has much lower hyphae counts than summer butter of the same grade.

Molds are not the main cause of flavor deterioration in cream and, since the flavor of butter is so largely determined by the flavor of the cream, no close correlation between mold content and flavor grade of butter can be expected. Some lots of cream having a very poor flavor contain very little mold and other lots having a much better flavor contain relatively large amounts. Mold growth is primarily at the surface of cream while deterioration occurs throughout the mass of cream.

Lots of cream having high mold contents show a variety of flavor defects, which suggests that mold growth was not the determining factor in the flavor deterioration; various bacteria are the most important organisms from the standpoint of off flavors in cream. Many lots of very high acid cream, which are certain to make low-grade butter, contain relatively little mold. Comparisons show that flavor deterioration in cream is much more rapid than the increase in mold content.

Different investigators have noted that cream held in buying stations may rapidly deteriorate in flavor without a significant change in mold content. For example, Morrison *et al.*[29] studied 113 lots of cream held in 10-gal cans in buying stations for 1 or 2 days at slightly below atmospheric temperature. The quality of cream, as indicated by grade and acidity, deteriorated rapidly while frequently the visual mold score did not reflect this deterioration.

In trials by Adams and Parfitt,[17] controlling the mold growth on a portion of a lot of cream by means of a layer of mineral oil did not result in a significant effect on the flavor of the butter made from it, compared to that of butter made from the cream on which mold growth occurred. Elliker [18] limited the growth of molds in cream by twice-a-day stirring but found that the flavor of the unstirred cream was unquestionably superior to that of the stirred cream; he suggested this may have been due to the larger numbers of yeasts and higher titrable acidities in the stirred cream.

Sources of organisms in fresh butter. The organisms in fresh butter come from various sources, the relative importance of the sources depending on the conditions of production. In a well-operated plant the cream that has been prepared for churning contributes most of the organisms in the butter; with insanitary methods many organisms come from other sources, especially the equipment.

Cream. Sweet cream used in the manufacture of butter varies widely in its microbiological condition. Although some of it is skimmed in plants and may contain comparatively few organisms, much of it is separated on farms and commonly is held there at temperatures that permit growth of organisms. Yeasts and molds regularly are present in sweet cream, although in some cases the numbers are very small. Macy *et al.*[30] studied 45 lots of sweet cream, each sufficient for a churning. Bacterial counts ranged from 1,520,000 to 160,000,000 per milliliter, with 77.3 per cent of the samples having counts over 10,000,000; yeast counts varied from 50 to 14,500 per milliliter, with 48.9 per cent of the samples having counts over 1,000; mold counts varied from 2 to

32,000 per milliliter, with 44.4 per cent of the samples having counts over 100.

Sour cream manufactured into butter inevitably contains large numbers of organisms, since many are required to produce the acid present. It regularly contains hundreds of millions of bacteria per milliliter. With moderate acidities *S. lactis* is the predominant species, and with high acidities both *S. lactis* and lactobacilli are prominent; the excessive acidities sometimes noted in cream are only possible through activity of the lactobacilli. Various other bacteria also are present, the number and species depending primarily on contamination of the cream and conditions of holding. Since yeasts and molds regularly are present in sweet cream, they also are found in sour cream in which they may have grown extensively.

In the pasteurization of cream intended for butter, exposures higher than those used for market milk often are employed and result in a high destruction of organisms. Many of the organisms present are easily killed by heat, particularly such types as *S. lactis* and *Pseudomonas* species.

With 45 samples of sweet cream studied by Macy *et al.*,[30] the cream was either heated to 65.6 C (150 F) and held for 30 min or heated to 62.8 C (145 F) and the temperature then raised to 73.9 C (165 F) over a period of 30 min. Under these conditions 94.20 to 99.99 per cent of the bacteria were destroyed, the percentages ranging from 99.0 to 99.9 with 93.2 per cent of the samples; counts after pasteurization ranged from 110 to 890,000 per milliliter. From 88.98 to 100 per cent of the yeasts were destroyed, all the yeasts being destroyed in 71.1 per cent of the samples; counts following pasteurization ranged from <1 to 540 per milliliter. After pasteurization the mold counts always were <1 per milliliter.

Destruction of organisms in sour cream by pasteurization regularly is high. The bacterial efficiencies commonly approximate 99 per cent and often are 99.9 per cent. Hunziker *et al.*[31] studied 33 lots of cream with 3 methods of pasteurization and found 99.88 per cent of the yeasts and molds destroyed at 62.8 C for 20 min, whereas 86.18 per cent were destroyed with flash pasteurization at 73.9 C, and 98.90 per cent with flash pasteurization at 85 C (185 F).

General results on the pasteurization of cream for butter indicate that destruction of the contained organisms normally is satisfactory. Defects that develop in butter as a result of activity of micro-organisms commonly are due to contamination of the pasteurized cream or the butter, rather than to organisms surviving the heat treatment.

Culture. When culture is used in the cream churned, most of the culture organisms are held in the buttermilk, but some of them are carried into the butter granules and appear in the finished product. If the culture is contaminated, the organisms contributed by it may include species that cause deterioration in butter.

Equipment. Vats, coolers, pumps, piping, etc., may be sources of contamination with cream intended for butter, and adequate methods of cleaning and sterilization are necessary. Churns constitute the characteristic equipment of butter plants, and without proper care they are an important source of the micro-organisms in butter. Metal churns have an advantage from the sanitary standpoint, but the usual types of churns still are made of wood. The surface of the wood is more or less irregular, the irregularities increase as the wood takes up water, and the wood may crack and sliver, so that removal of organisms and milk solids is difficult. The heavy loads that churns must carry and the strain thrown on them during working of butter tend to open joints in the wood, loosen bolts, etc., and thus further increase the difficulty. When churns were improved mechanically by building into them the equipment for working butter, they became more of a problem from the sanitary standpoint.

A churn can be studied for the numbers of organisms contained by adding sterile water, operating it for a short period, and then plating the water, but with this procedure many organisms are not removed, especially from inaccessible points. The agar disc method [32] also can be used, but the organisms are difficult to remove from a wood surface and discs cannot be prepared at the locations where the contamination probably is heaviest.

Various investigators have emphasized the importance of churns as a source of organisms in butter and also the difficulty of keeping churns in a satisfactory microbiological condition. Libbert [33] isolated 57 yeast and mold cultures from various churns and all except 3 of the yeast cultures grew on a medium containing water, agar, and ground fir wood. The organisms also grew on the medium minus the fir wood but such cultures died in 10 days, whereas those on the medium containing fir wood remained viable for at least 6 weeks. By dismantling 2 churns which had been in service for a number of years and culturing samples of scrapings, wood, etc., Macy *et al.*[34] demonstrated that numerous contamination foci exist in a churn. Of 230 samples from a churn receiving ordinary treatment, 192 yielded molds; of 73 samples from a churn receiving careful treatment, only 22 yielded molds and, in general, the contamination was not heavy. Yeasts were present in the majority of the samples and bacteria always were present, sometimes in large numbers. With the agar disc method Olson and Hammer[32] found that 27 churns in commercial use in 24 plants varied widely in their microbiological condition, some of them containing comparatively small numbers of organisms and others containing excessive numbers. Bacteria regularly were much more numerous in the churns than yeasts or molds, and molds usually were somewhat more numerous than yeasts. When the bacterial counts were low, comparatively few species were present and *Bacillus* and *Micrococcus* organisms predominated; when the counts were high many species commonly were present. In studies on *Ps. putrefaciens,* Long and Hammer[35] isolated it and also other species, including yeasts and molds, from various points in churns which were reached by loosening bolts, dismantling parts, etc.

The first churning of the day from a churn sometimes shows a higher content of bacteria, yeasts, and molds than the second churning, even when cream from the same lot is used. This is due to elimination of many of the organisms from a contaminated churn during the period of use and is convincing evidence of the importance of churn sanitation in controlling the microbiological condition of butter.

The principles involved in the proper care of a churn are the same as in the care of other equipment. The churn should be thoroughly cleaned, then most of the organisms remaining should be killed, and finally the churn should be left in a dry condition to prevent growth of organisms in it. A general procedure for the care of churns is as follows: Immediately after use the milk solids are rinsed from the churn by adding water at a temperature of 37.8 to 48.9 C (100 to 120 F), revolving for several minutes, and then draining. The churn next is filled one-third to one-half full of water at 76.7 to 82.2 C (170 to 180 F), washing reagent added, and the churn revolved in high gear for 15 min and drained. The churn then is filled one-half full of water at 93.3 C (200 F), or higher, and revolved in high gear for 15 to 20 min; it is drained thoroughly, allowed to stand with the doors up for a few minutes, again drained, and then again left with the doors up. As soon as the churn is dry it is moved so the doors are about two-thirds of the way up. Each door is covered with a frame carrying wire screen or muslin. Occasionally, about 1 lb of slaked lime per 100 gal of water is added to the last wash water. Various investigators have emphasized the necessity of using large volumes of very hot water in a churn in order to heat the wood, which transfers heat only slowly; thorough heating is necessary to kill organisms and also to facilitate rapid drying of the churn. Before use a churn is rinsed by filling it one-third to one-half full of cold water, revolving in high gear for a few minutes, and then draining. In some cases a chlorine rinse is used in a churn. Preferably, this is employed just before the churn is put into operation.

Churns also can be heat treated with steam. Cordes [36] reported that steam treatment for 30 min, when used daily, left a churn barrel in a reasonably satisfactory condition.

In some instances plant operators fail to treat a churn properly with moist heat because of the effect on the wood. However, adequate destruction of the organisms left in a churn after use, together with proper drying of a churn, are essential in the modern manufacture of butter, and such destruction and drying can best be accomplished with hot water or steam. Quality of the wood used is an important factor in the life of a churn. In some instances one or more pieces of wood in a churn deteriorate very rapidly, regardless of the churn treatment. Many churns made from good-quality wood have been in use over extended periods with adequate heat treatment.

Wash water. Wash water is a source of organisms in the butter on which it is used. With an adequate supply the numbers and types of organisms from this source are of no significance, whereas with contaminated supplies organisms added to the butter may be the cause of serious deterioration. Many outbreaks of rapid deterioration in butter have been traced to contaminated water supplies (Chapter 18).

Air. Air contamination is more important in a butter plant than in other dairy plants because butter often is exposed rather extensively during the packing, it frequently is held for relatively long periods at temperatures permitting growth of organisms, and development of molds on butter is a serious defect.

Olson and Hammer [37] studied the numbers of bacteria, yeasts, and molds falling from the air by exposing agar plates once a week for approximately 1 yr at six locations, three in dairy plants, one in a bacteriological laboratory, and two out-of-doors. They found that, in general, the three groups of organisms were rather constantly falling, bacteria being the most numerous and yeasts the least numerous. The numbers of micro-organisms falling indoors did not differ greatly from the numbers falling out-of-doors, and there was no distinct seasonal variation. A muslin cover reduced the number of organisms falling on a surface. The results showed the constant danger of organisms falling on the surface of exposed butter; undoubtedly, molds constitute the most serious contamination from the air.

Butter color. Jamieson and Chen [38] made plate counts on butter color and found that, as it comes from the manufacturer, it is practically free of micro-organisms. It may be contaminated in the plants by holding in opened containers. It also may pick up bacteria, yeasts, and molds from the lip over which it is poured; such contamination was greater with containers stored in open creamery rooms than with those stored in protecting cupboards. Counts on 8 samples of color from supplies in regular use, after pouring over the top of the can or a special lip, were 2,400 to 80,000,000 total bacteria per milliliter, <1 to $>10,000$ coliform bacteria, 90 to 180,000 yeasts, and <1 to 1,400 molds. Unwashed measuring glasses also may contaminate the color. There was no conclusive evidence of growth of organisms in the product. A large proportion of the bacteria and some of the yeasts were types that are detrimental to butter quality.

Plant employees. Persons making and handling butter may add organisms to the cream used and to the finished butter. They come from the hands, mouth, nasal passages, and clothing. Although the numbers from these sources are small, they may be of importance under certain conditions. Molds carried to the finished butter as a result of handling raw cream, cans, etc., may be especially serious from the standpoint of mold growth in butter.

Packaging materials. Unless wrappers, liners, circles, etc., are properly handled they may be sources of organisms, the greatest danger probably involving addition of molds to the butter. Ordinarily, these materials come from the manufacturer in a satisfactory microbiological condition, but they may be contaminated in the plants if not properly protected. They can be treated with hot water, hot salt solution, or a chemical solution if treatment is considered necessary. Because of use of poor storage conditions, green material, etc., wood tubs and boxes sometimes are heavily contaminated with organisms. They can be treated with hot water or steam. When butter tubs or boxes are being reused, special care in cleaning and reconditioning is advisable.

Comparative numbers of organisms in cream and butter. When cream is churned the great majority of the organisms present in the cream are carried into the buttermilk so that fresh butter commonly contains a smaller number per milliliter than the cream from which it came. Grimes[80] reported that butter made from cream in which culture was used retained about 0.5 to 2.0 per cent as many bacteria per milliliter as were present in the cream; with sweet cream containing no culture the values were about 5 to 30 per cent.

Data of Macy *et al.*[30] also show that the bacterial count per milliliter of butter commonly was lower than that of the cream in the churn. However, the yeast and the mold counts often were higher on the butter than on the cream; this was attributed to contamination from remote parts of the churn, and in the case of the molds to the breaking up of aggregates of conidia.

Parfitt[40] inoculated pure cultures of yeasts and bacteria into sterile cream and then churned the cream in sterile jars. Three species of yeasts (*T. cremoris* and 2 unidentified) and 2 species of bacteria (*Ach. lipolyticum* and *A. aerogenes*) were used. In 14 trials the numbers of yeasts varied from 4,700 to 800,000 per milliliter of cream, and in 8 trials the numbers of bacteria ranged from 28,000 to 750,000. The numbers of yeasts in the buttermilk varied from 2,600 to 2,000,000 per milliliter, and the numbers of bacteria from 40,000 to 600,000. The numbers of yeasts were higher in the buttermilk than in the cream in 9 trials, whereas the numbers of bacteria were higher in the buttermilk than in the cream in 5 trials. The wash water removed considerable numbers of organisms. The butter was not salted and the numbers of organisms per milliliter in it always were smaller than the numbers in the cream; the yeast counts varied from 180 to 190,000 per milliliter and the bacterial counts from 100 to 42,000. With the yeasts the counts on the butter were 1.8 to 27.3 per cent (avg 13.4%) of the counts on the cream; with the bacteria the percentages ranged from 0.3 to 10.5 (avg 3.9%).

Elliker[41] found that during the churning of cream to which culture had been added bacteria tended to go into the buttermilk while many more were removed by the wash water. For example, in one comparison direct microscopic counts were butter 8,660,000 per gram; buttermilk 415,680,000; and wash water 25,114,000. Percentages of the total bacteria in the different fractions were butter 0.75; buttermilk 91.57; and wash water 7.68.

Elimination of large numbers of organisms in the buttermilk undoubtedly is a desirable thing from the standpoint of the deterioration of butter since it results in contamination of only a relatively small percentage of moisture droplets.

Numbers of organisms in fresh butter. The bacterial content of fresh butter is extremely variable and depends primarily on the methods of manufacture. Although sweet cream contains fewer organisms than sour cream, pasteurization largely eliminates the original difference, and there is no regular variation between the bacterial counts on sweet-cream and sour-cream butter. When culture is used, either with or without actual ripening of the cream, the numbers of bacteria in the cream and in the butter made from it are greatly increased. Ordinarily, use of culture greatly overshadows extensive contamination from equipment as far as numbers of bacteria are concerned; however, species of organisms from the two sources differ strikingly. The bacterial content of fresh butter varies from a few thousand per milliliter, in the case of a product properly made from thoroughly pasteurized cream without use of culture, to several million when culture is employed.

The numbers of yeasts and molds in butter also vary widely. Some samples may fail to show any of these forms in 1 ml; counts on others run into the hundreds of thousands. Ordinarily, when the yeast and mold count is high yeasts are much more numerous than molds.

Factors influencing growth of organisms in butter. A number of factors definitely influence growth of organisms in butter. Apparently, these vary in their effects on different species and a factor which largely prevents growth of one species may have little influence on another. Hammer and Long[42] noted that some of the factors which tend to restrain growth of bacteria in butter are very effective if completely operative, and the action of two, or more, of them in combination would be expected to control bacterial action in butter. However, there are counterinfluences so that the actual growth of bacteria in butter is determined by the balance of the factors restraining it and those favoring it.

Physical condition. Various investigators have shown that the water in butter is divided into enormous numbers of tiny droplets, roughly from 10 to 18 billion per gram. This distribution is of great significance from the standpoint of growth of organisms in butter. Rahn and Boysen[43] consider that a portion of the moisture carried from the cream into the butter becomes sterile through division into very tiny droplets. The number of bacteria per gram is much smaller than the number of moisture droplets, so that most of the moisture droplets are left in a sterile condition. Rahn and Boysen noted that the amount of moisture from which bacteria are excluded depends on two factors, number of bacteria in the cream at the moment of churning and degree of dispersion of the moisture in the butter. They found that formation of acid in the moisture within butter is much slower than if the same amount of moisture is freed of fat and is in the form of a continuous liquid. However, the percentage of acid slowly formed apparently increases above the theoretical value computed from the amount of moisture that is infected. This is accounted for by diffusion of acid from infected droplets to those free of bacteria. Rahn and Boysen consider this diffusion is probable because the distance between moisture droplets averages not more than 5 microns, and diffusion of salt into unsalted butter has been established. Overworked butter showed less diffusion.

Hammer and Hussong[44] found that multiplication of organisms in unsalted butter was more rapid when the serum was separated than when the butter was left in a normal physical condition. This indicates that, with the serum separated, the nutrients contained are more readily available for the organisms present. With salted butter separation of the serum increased the rate of destruction of organisms.

Using an adaptation of the Burri technic, Long and Hammer[6] examined very small portions of butter for their bacterial contents and noted an irregular distribution which, in many instances, was extreme. This was considered additional evidence that bacterial growth in butter is largely limited to certain focal points, probably infected water droplets.

Scharp[45] concluded that as the working of commercial unsalted butter is increased the number of organisms after any holding period decreases; this was attributed to finer dispersion of the water. As judged by rate of growth, production of defects, production of acid by culture organisms, and liberation of fatty acids by lipolytic bacteria, Long and Hammer[46] found that bacteria are more active in underworked than in thoroughly worked butter. Various investigators have noted that thorough working tends to retard appearance of the putrid defect in butter.

Reworking butter may have an effect on bacterial growth in it through the influence on moisture distribution. Practical experience indicates that butter which has been through a printer having a reworking effect sometimes deteriorates rapidly, whereas unprinted butter from the same churning keeps satisfactorily. Apparently, redistribution of organisms and aggregation of water and the nutrients contained in it are responsible for the increased bacterial activity. Cullity and Griffin[47] reported cases in which reworking of apparently sound butter rapidly resulted in the putrid defect. Turgasen[48] cited instances in which butter printed in a printer having a reworking effect became cheesy, whereas unprinted butter from the same churning did not. Long and Hammer[49] concluded that reworking unsalted butter, which was made from pasteurized cream inoculated with various organisms, frequently increased the activity of organisms at 10 C (50 F) as evidenced by growth rates, production of defects, decreases in *p*H of the butter serum when cultures were used in the cream, and increases in fat acidities when lipolytic organisms were used. Apparently, redistribution of organisms and aggregation of water and the nutrients contained in it were primarily responsible for the increased activity.

Collins and Hammer[50] studied migration of bacteria through unsalted butter held at temperatures favorable for growth and noted that it was not common. This was the case both when the organisms were added from agar or milk cultures and when butter that had been inoculated by adding organisms to the cream from which it was churned was placed in contact with uninoculated butter. In the few instances in which migration occurred, poorly worked butter was involved more often than well-worked butter. The distances bacteria migrated through butter always were comparatively small, but they sometimes migrated extensively along the wall of the container in which the butter was packed, and also through water held at the surface of butter by parchment. It appeared that extensive migration along the wall of a container was due to collection of water there, and that migration through butter was largely due to water channels in the product.

It is evident that the physical condition of butter is an important factor in restraining growth of organisms in the product and is of significance from the standpoint of preventing deterioration in butter. Organisms that can cause deterioration must be present in relatively large numbers if sufficient moisture droplets are to be infected to influence the product significantly. However, even with this restraining effect very large numbers of organisms sometimes are present in butter held under conditions that are favorable for growth. Counts in the hundreds of millions or even more than a billion per milliliter have been noted with organisms that find butter well adapted to their development.

In considering the moisture in butter, it should be recognized that there are differences in the opportunities for the dispersion of moisture from different sources. Presumably, the opportunities are best with the moisture coming from the cream, somewhat less with moisture from the butter wash water, and least with moisture added late in the working of the butter to adjust the moisture content. Moreover, water from the cream probably contains more milk solids that would favor growth of organisms. These relationships may be of significance from the standpoint of bacterial growth in butter.

It appears that moisture dispersion in butter is of much less significance in the growth of molds and yeasts in the product than in the growth of bacteria.

Salt content. Salt tends to retard bacterial growth in butter and thus delays or prevents bacterial spoilage; apparently, it sometimes destroys organisms. Action of salt is influenced by concentration in the water droplets and by uniformity of distribution in them. Even with considerable salt in butter the concentration in certain droplets may not be sufficient to inhibit all bacterial growth. Instances have been reported in which a species relatively sensitive to salt has produced a conspicuous defect in commercial salted butter, presumably because of poor salt distribution. Washburn and Dahlberg [51] noted that in unsalted butter bacteria increased more rapidly at 14.4 C (58 F) than in salted butter; but they decreased more rapidly at −26.1 C (−15 F). Hammer and Hussong [44] found that, although unsalted butter from a number of churnings increased in bacterial content at 21 C (69.8 F) or 7 C (44.6 F), salted butter from the same churnings tended to decrease at these temperatures. Salt also delays or prevents growth of various yeasts and molds in butter. The general effect of salt on butter deterioration due to organisms is regularly recognized.

The presence of salt lowers the freezing point of butter so that organisms may grow at temperatures below 0 C (32 F). Rahn *et al.*[52] reported that some organisms multiplied in salted butter at −6 C (21.2 F). However, at such a temperature growth of organisms is limited by both the temperature and the salt.

The influence of salt on growth of bacteria in butter varies greatly with the species present. In experimental churnings made from lots of cream inoculated with various organisms causing defects in butter, it commonly is noted that deterioration is prevented in some of the butter by salting, whereas when other organisms are involved deterioration is not prevented, although it may be delayed. Under commercial conditions salted butter sometimes shows conspicuous bacterial deterioration so that the presence of salt is no assurance that bacterial action will be controlled.

Nelson and Hammer [53] found that, in general, lactic acid culture streptococci developed little or not at all in salted butter held at favorable growth temperatures, whereas in unsalted butter they developed extensively, as was evident from both plate and microscopic counts; the most striking evidence of growth was the appearance of long chains in the butter.

Jacobsen [54] found with 15 churnings, from which both salted and unsalted butter were prepared, that the original counts were lower with the salted butter, presumably because of the destructive action of the salt. Holding at −31.7 C (−25 F) resulted in a marked decrease in total numbers of bacteria with each type of butter; the decrease was smaller with the salted product.

Studies on the distribution of salt in butter on a macro basis have given information that is useful in composition control, etc., but, from the standpoint of bacterial action in butter, values on much smaller portions are desirable. Hoecker and Hammer [55] studied salt and moisture distribution in butter with samples that commonly were less than 1 mg, these being the smallest portions giving satisfactory analytical results. In some samples of normal butter the salt was uniformly distributed and in others it was not; with most samples there was a correlation between salt distribution and moisture incorporation. In general, as the working of butter continued the salt became more uniformly distributed. Addition of 1.5 per cent salt greatly inhibited bacterial action in experimental butter held at 15.5 C (59.9 F). Growth of various organisms in butter churned from inoculated, pasteurized cream was influenced by the distribution of salt in the butter, the lowest bacterial counts being obtained on butter having the salt well distributed. As determined by an adaptation of the Burri technic, the numbers of organisms were lower and they were more uniformly distributed in experimental butter containing organisms capable of producing a defect when the salt was well dispersed than when it was poorly dispersed. When lots of pasteurized cream, each inoculated with an organism capable of producing a defect in butter, were churned and the butter held at 15.5 C, the butter with the salt well distributed usually did not develop defects, whereas the butter with the salt poorly distributed frequently did. Both thoroughly and poorly worked unsalted butter usually developed defects; they commonly appeared earlier in the poorly worked butter.

In considering the influence of salt on the organisms in butter it should be recognized that the percentages of salt in various lots of butter differ widely so that there may be a greater variation in salt content between two lots of salted butter than between certain lots of unsalted butter and low-salted butter. With this situation prevailing, information on the amount of salt present has a definite microbiological significance.

Temperature. Low temperatures constitute one of the important factors in the control of bacteria in butter. Data of various investigators indicate that at 0 C (32 F), or below, growth of bacteria largely is prevented and that some decrease in numbers may occur. Above 0 C bacterial development may be rapid, especially in unsalted butter; commonly, growth is more rapid at higher temperatures than at 0 C. When unsalted butter is made from cream containing an organism capable of causing a defect and different portions are held at temperatures ranging from 0 to 21.1 C (32 to 70 F), the period required for the defect to develop increases as the temperature decreases. When bacterial growth occurs in salted butter, the same relationship probably obtains.

At commercial storage temperatures, such as −17.8 C (0 F) or lower, organisms do not increase in butter, whether it is salted or unsalted, and there may be a decrease in numbers. The defects that develop are due primarily to chemical action.

Arup and Gilmour [56] stored salted butter at approximately −7 C (19.4 F) for 6 mo. There was little or no increase in yeasts, molds, or bacteria, and no considerable reduction in counts occurred. Later, the investigators [57] stored butter at −2 C (28.4 F), −6 C (21.2 F), and −12 C (10.4 F) for 6 mo. Growth of micro-organisms was stopped at all three temperatures. At −2 C yeast counts after storage indicated very little mortality; at the lower temperatures considerable reduction in counts occurred. Although molds were absent from many of the samples of butter the counts indicated little or no growth at −2 or −6 C, but there was a reduction at −12 C. Bacterial counts were reduced at all 3 temperatures. Loftus-Hills *et al.*[58] stored salted butter at −11.1 C (12 F) for 3 mo; there were only slight changes in bacterial counts during the cold-storage period.

Freezing butter known to be susceptible to rapid deterioration does not give a product that will keep when placed at temperatures favorable for deterioration. Turgasen [48] investigated the effect of extended holding of butter at −28.9 C (−20 F) on activity of organisms causing cheesiness; infected print butter stored for 6 mo developed the defect when placed at 21.1 C (70 F). Jacobsen [54] found, with unsalted butter, that holding at 21 C after a storage period resulted in more rapid increases in bacteria and in quicker flavor deterioration than occurred when the fresh butter was held 7 days at 21 C. With salted butter, the bacterial counts did not change significantly in 7 days at 21 C following the storage.

Acidity. Various organisms are sensitive to acid and fail to grow in acid dairy products. In butter, low *p*H values presumably limit or prevent growth of acid-sensitive organisms and various investigators have emphasized their restraining action. With salted butter the *p*H commonly is kept relatively high because of the influence of acid on certain types of chemical deterioration in butter, but with unsalted, ripened-cream butter the *p*H commonly is relatively low and definitely may restrain certain bacteria.

In laboratory trials it has been found that organisms responsible for the putrid defect in butter can be limited in their growth by use of lactic acid culture in the cream. On the other hand butter made with culture definitely has developed the defect under practical conditions. It may be that the source of the organisms is an important factor. Organisms in the cream to which the culture was added might be restrained much more than organisms added with the wash water; presumably, the latter would be subjected to the acid much less than the former. There is some evidence that the culture organisms may have a restraining effect in addition to that of the acid.

The protective action of acid alone may be overemphasized, particularly in the range of practical limits as fixed by flavor, keeping qualities, etc., of the butter. Gilmour and Cruess-Callaghan [59] could detect no relationship between acidities of Irish Free State creamery butter and rates of growth of micro-organisms as a group. Within experimental limits, Pont and Sutton [60] observed no correlation between *p*H values and bacterial counts of New South Wales butter.

Air supply. Butter held under conditions permitting growth of organisms often shows higher counts at or near the surface than in the interior, and certain microbiological defects of butter commonly are first evident at the surface. In general, these relationships are interpreted as effects of the air supply on growth of organisms. Extensive growth of organisms in butter ordinarily is followed by a decrease in numbers so that with rather extended holding the count at the surface may decrease to a point where it is lower than the count at the interior. For example, Hammer and Collins [61] found that lipolytic bacteria commonly grew faster at the surface of experimental butter than in the deeper layers and early in the holding were more numerous at the surface; later, the larger numbers sometimes were found in the interior, because of a decrease in numbers at the surface.

The putrid defect of butter normally is first evident at the surface of a print or tub and then later is detected in the interior; presumably, this is due to the effect of the air supply at the surface on growth rate of the organisms.

General considerations. Although there are various factors tending to restrain growth of bacteria in butter, increases in numbers and spoilage due to bacterial growth are common in the commercial product; in many instances the defects are so inconspicuous they are noted only by the critical consumer but in other cases they are extremely objectionable. For each restraining factor there are counterinfluences and in some instances these outweigh the restraining effect. Hammer and

Long[42] outlined the factors limiting growth and the counterinfluences as follows:

FACTORS LIMITING BACTERIAL GROWTH IN BUTTER AND COUNTERINFLUENCES

Factors Limiting Growth	*Suggested Counterinfluences*
Fine dispersion of water	High initial contamination resulting in many infected water droplets Printing butter under conditions tending to aggregate water droplets Action of salt in tending to aggregate water droplets
Addition of salt	Poor distribution of salt Relatively little salt added High initial contamination Presence of salt-tolerant species Adaptation of certain species to relatively high salt concentration
Low temperature	Presence of psychrophilic organisms that grow just above freezing point of water Exposure of butter for short periods (during handling, etc.) to temperatures allowing rapid growth Depression of freezing point of water in butter by salt
Use of lactic acid culture; acidity	*S. lactis* less salt-tolerant than many other species Inhibition of *S. lactis* but not psychrophilic species by holding temperatures Acid content of salted butter and some unsalted butter commonly too low to inhibit organisms
Air supply	Air incorporated in butter during manufacture sufficient for many species

Effect of growth of organisms in butter. Many species of organisms grow extensively in butter without significantly influencing the flavor, and as a result certain lots of butter with very high counts show no deterioration that can be attributed to organisms. In general, such species also bring about changes in milk slowly, even when the numbers per milliliter are large. Other species produce changes in butter quickly when conditions for growth are favorable and these organisms are important in certain defects that develop in butter; commonly, changes in milk also are brought about rapidly.

Certain general types of organisms are of special importance in the deterioration of butter. For the most part they are capable of growth at relatively low temperatures and may fail to grow at 37 C (98.6 F); also, they are types that attack protein and/or fat and often have little effect on lactose.

Determination of the effect of an organism on butter. Study of the effect of an organism on butter presents difficulties not encountered with milk or cream in which the moisture is the continuous phase. Inoculation of an organism into butter is not satisfactory. The organism must be well distributed through the product, which is best accomplished by inoculating it into cream and then churning. Ordinarily, experimental churnings involving from one to several pints of sterilized or thoroughly pasteurized cream are used. Sterilization gives the cream and butter such a conspicuous heated flavor that detection of a defect often is difficult. Moreover, experience indicates that organisms surviving adequate pasteurization rarely cause flavor defects in butter. Precautions against contamination are advisable and should include use of sterile wash water, butter-working equipment, and containers. Some of the butter can be salted and some left unsalted if the influence of salt is of interest, and portions can be packed for storage at different temperatures. Comparisons of the butter churned from inoculated cream with that churned from uninoculated cream should be made frequently, since defects sometimes become conspicuous and then tend to change in character or become less pronounced.

SOURCES OF THE FLAVOR OF BUTTER

The general flavor of butter is the most important quality from the standpoint of market value of the product. It involves flavors from various sources and what the butter grader evaluates is a combination of flavors. These can be divided into two groups, (1) the desirable flavors, and (2) the undesirable flavors.

DESIRABLE FLAVORS OF BUTTER

The desirable flavors of butter come from two sources, the milk constituents and the products of bacterial growth.

Milk constituents. Various milk constituents influence the flavor of butter. The fat is of most importance in this connection because of the percentage in which it is present. The flavor of the fat of milk is more or less characteristic and differs from the flavors of other edible fats, such as those used in margarine. Butter churned from cream in which there has been no development of organisms has a definite butter flavor that is very pleasing and is the type of butter desired in certain markets, but it lacks the high flavor, preferred by some critical consumers, which is present only in butter made so that it contains products formed through action of certain bacteria.

Products of bacterial growth. During the natural souring of milk or cream, compounds commonly are produced that give butter a desirable flavor but under these conditions objectionable compounds also are formed so frequently that cultures known to give a desirable flavor are employed in manufacturing butter. Usually, lactic acid cultures are used but various special cultures also have been suggested. Because of its importance, action of lactic acid cultures in butter has been extensively investigated (Babel and Hammer [62]).

Normally, in the manufacture of salted butter the culture is (1) added to the pasteurized cream at churning, (2) added to the pasteurized cream and the mixture held cold for some hours, (3) added to the pasteurized cream and the mixture ripened somewhat, or (4) added directly to the butter. Each procedure has its advantages and disadvantages. Addition of culture to cream carries flavor materials into the cream or butter. With holding or actual ripening, there may be a production of flavor materials in the cream. In the manufacture of unsalted butter with culture, the cream ordinarily is ripened extensively so that relatively large amounts of flavor materials are produced.

The chemical compounds involved in giving butter a desirable flavor through the use of culture are volatile acids, biacetyl, and perhaps others; these are the compounds that give lactic acid cultures a pleasing flavor and, actually, such cultures first were selected on the basis of their value in giving butter a desirable flavor.

Volatile acids give butter a pleasing, slightly acid aroma; on the basis of the products formed in lactic acid cultures, acetic acid is of primary importance. Addition of acetic acid in the proper amount to sweet cream that is churned into butter without use of culture gives a pleasing flavor to the product, but it is not a typical culture flavor. Excessive acid aroma in butter is objectionable, and butter graders criticize it severely.

The studies of van Niel *et al.*[63] indicate that biacetyl is a flavor contributant of butter. In fine butter 0.0002 to 0.0004 per cent biacetyl was found and, when these concentrations were added to butter neutral in odor, an unmistakable aroma appeared. Schmalfuss and Barthmeyer [64] noted the presence of biacetyl in various materials, including butter. Margarine to which biacetyl was added had the aroma of butter. In a number of samples of butter examined, the biacetyl content was correlated with the intensity of flavor.

Various analyses and many trials in which biacetyl has been added to butter indicate that the compound definitely contributes to the flavor of the product. The flavor concentrates that have been developed for butter, and for products intended to have the flavor of butter, contain primarily biacetyl although other flavor materials also may be included. There is no close correlation between the biacetyl content and score of butter because off flavors that definitely lower the score cannot be covered by any normal amount of biacetyl and excessive amounts give butter an artificial flavor that is objectionable. Although some consumers prefer butter that is lacking in biacetyl flavor, many others prefer such a flavor and it is essential in unsalted butter for table use.

Biacetyl has been found important in the food preferences of animals. Deuel and Movitt [65] noted that rats preferred a diet of mineralized vitamin-fortified skim milk powder containing butter to one containing other fats (including margarine), apparently because of flavor. Animals preferred a margarine or peanut oil diet containing 4 ppm biacetyl to an unflavored diet; they also preferred this level of biacetyl to a higher one (8, 16, or 24 ppm). Commercial butter flavor was preferred to biacetyl; a laboratory-prepared butter flavor containing monobutyrin, butyric acid, and biacetyl gave similar results. The authors concluded that flavor may play an important role in determining food consumption of diets that are satisfactory from a nutritional standpoint.

Factors influencing biacetyl content of butter. The biacetyl content of butter is influenced by various factors; these begin with quality of the cream and continue through washing of the butter. Commonly, a high or low biacetyl content accompanies a high or low acetylmethylcarbinol content so that a factor favoring biacetyl production also favors production of the carbinol.

Quality of cream. It appears that perfectly sweet cream may contain biacetyl and acetylmethylcarbinol; presumably, the amounts are influenced by the feed. During the souring of cream these compounds commonly are produced, often in relatively large amounts, but objectionable flavor compounds frequently develop also and these make the cream less satisfactory for butter manufacture than it was before the souring.

Neutralization and pasteurization of cream. According to Barnicoat,[66] neutralization and pasteurization of cream caused no notable decrease in biacetyl but a distinct decrease in acetylmethylcarbinol. Destruction of the carbinol was correlated with the neutralization point of the cream. Davies [67] noted that cream which had developed biacetyl and acetylmethylcarbinol did not yield butter having flavor and aroma when the cream was neutralized and flash pasteurized. Disappearance of the compounds from the cream was explained on the basis of their volatility and absence of oxidation of the carbinol in the butter. Prill and Hammer[68] found marked changes in biacetyl contents, both decreases and increases, during neutralization and pasteurization of sour cream; changes in the carbinol were relatively small. It appears that generalizations on the effect of neutralization and pasteurization of the cream on acetylmethylcarbinol and biacetyl cannot be made because of variations in the original cream and in the neutralizing and pasteurizing procedures.

Use of culture. Use of culture in the cream increases the biacetyl and acetylmethylcarbinol contents of butter. Cultures show wide variations in quality and some definitely are more desirable than others. Also, a culture may vary from day to day so that constant checking on the quality is essential. The acidity developed in cream greatly influences the production of biacetyl and the carbinol and, if there were no other considerations, a low *p*H would be advisable. However, with salted butter, a low *p*H in the cream favors certain chemical changes in the product and the *p*H must be kept relatively high if these are to be avoided. With unsalted butter a high flavor can be obtained by ripening the cream to a low *p*H. Temperature influences the ripening of cream but the studies along this line do not justify conclusions that can be applied practically. It appears that there may be definite development of biacetyl and acetylmethylcarbinol during the period a mixture of cream and culture is held cold preparatory to churning but that with extended holding an increase is followed by a decrease.

Addition of citric acid. The effect of added citric acid in increasing the biacetyl and acetylmethylcarbinol contents of lactic acid cultures indicates that under certain conditions its addition to cream intended for butter would be useful. Added citric acid appears to be of special value in the manufacture of unsalted butter from sour cream since the bacteria in sour cream often have lowered the citric acid content from that originally present.

Aeration. The influence of oxygen in the fermentation of citric acid by culture organisms indicates that a good oxygen supply would favor biacetyl production in cream.

Agitation during churning. The agitation of cream and butter granules in the presence of air during churning would be expected to have an effect on the biacetyl and the carbinol contents. Mazé[69] considered that churning must be regarded as an additional aerobic fermentation that would tend to oxidize the products of the anaerobic fermentation and produce the flavor characteristic of first-quality butter. Virtanen[70] stated that churning of cream in a churn filled with inert gas obviously is not suited to production of butter with a rich aroma; he suggested churning in such a manner as to ensure a maximum content of air in the butter, since this promotes formation of acetylmethylcarbinol and biacetyl. Analyses of Prill and Hammer[68] showed an increase in biacetyl during churning.

Partition of biacetyl and acetylmethylcarbinol during churning. Butter contains much less biacetyl and acetylmethylcarbinol than the cream from which it was churned and the buttermilk contains relatively large amounts. Percentage retentions in the butter reported by different investigators vary rather widely and variations also occur in tests by individual investigators, perhaps due to variations in conditions, especially the amount of buttermilk held in the butter.

Barnicoat[66] reported that 0 to 33 per cent (avg 15%) of the biacetyl and 7 to 22 per cent (avg 14%) of the carbinol in the cream were retained in the butter. Prill and Hammer[68] found that on the average 8.8 per cent of the biacetyl and 4.5 per cent of acetylmethylcarbinol plus biacetyl in the churning mixture were retained in the butter.

Washing of butter. Various investigators have noted that during washing butter loses flavor and that this is reflected in the biacetyl content of the butter and of the wash water. Prill and Hammer[68] found that butter wash water contained only a fraction of a milligram of biacetyl and acetylmethylcarbinol per kilogram. They considered that while the total content of these compounds in wash water may be significant, compared to that present in butter, it is difficult to differentiate between material that comes from the film of buttermilk remaining between the granules and that which might be removed from the granules themselves.

Partition of biacetyl and acetylmethylcarbinol between fat and serum of butter. Because of the comparative amounts of biacetyl and acetylmethylcarbinol in butter and buttermilk, the serum of butter would be expected to contain larger amounts of the compounds than the fat. Various investigations confirm this relationship. Hoecker and Hammer[71] reported that the serum of butter contains higher concentrations of the compounds than the fat, with the greater difference involving the carbinol. The percentages of biacetyl and acetylmethylcarbinol in the fat were smaller with unsalted than with salted butter. With biacetyl the percentage in the fat was independent of the concentration in the butter, whereas with acetylmethylcarbinol it tended to decrease as the concentration in the butter increased.

Changes in biacetyl and acetylmethylcarbinol contents during holding of butter. During the holding of butter there are various possibilities in connection with changes in biacetyl and acetylmethylcarbinol contents, such as increases through action of culture organisms and decreases through action of bacteria or chemical reactions. Various factors influence these possibilities and may explain the variations from one lot of butter to another.

Culture organisms are sensitive to salt and, with appreciable salt concentrations in butter, they probably are not active. However, there may be points at which there is little or no salt and here the organisms may be active. This could account for the increase in biacetyl in fresh salted butter that has been reported by certain investigators. Commonly, the *p*H of salted butter is too high for significant biacetyl production. In salted butter biacetyl and acetylmethylcarbinol seem to be rather well retained and a less satisfactory flavor on holding may be due to development of undesirable flavors rather than to loss of biacetyl.

In the case of unsalted butter made with extensive ripening of the cream, significant changes in biacetyl and acetylmethylcarbinol occur at temperatures permitting growth of the culture organisms. Both increases and decreases have been noted. Various factors undoubtedly are involved, such as *p*H, presence of citric acid, activity of culture organisms, etc. It appears that an increase in biacetyl content of unsalted culture butter is important in the flavor development which this type of butter often shows.

Prill and Hammer [72] noted that, in general, salted butter (about 2.25%) made with different manufacturing procedures showed only slight changes in biacetyl and acetylmethylcarbinol contents when held frozen or at 2.2 to 7.2 C (36 to 45 F). Even at 21.1 C (70 F), at which temperature chemical changes in butter are especially rapid, the compounds were largely retained for considerable periods. With unsalted butter in a frozen condition changes in the contents of the compounds were relatively slight but at 2.2 to 7.2 C or 21.1 C significant changes occurred, both increases and decreases being involved.

UNDESIRABLE FLAVORS IN BUTTER

Undesirable flavors in butter can be divided into two groups from the standpoint of the time of appearance: those present in fresh butter, and those that develop on holding. Various factors are responsible for these flavors but the products formed by organisms, either in the cream or in the butter, are directly or indirectly concerned with a number of them. The quantities of various chemical compounds required to influence the flavor of butter often are extremely small, which suggests that the changes resulting in their development may be slight.

The difficulty in determining the nature of these slight changes greatly complicates the whole problem of flavor defects in butter.

Causes of undesirable flavors in fresh butter. The flavors in fresh butter are closely related to the flavors in the cream as it goes to the churn. Certain flavors are more pronounced in the butter than in the cream through their association with the fat; others are less pronounced because they are chiefly associated with the non-fatty constituents and are largely carried away in the buttermilk. Washing of the butter tends to decrease the intensity of some flavors. Addition of salt brings out many flavors in butter so that certain undesirable flavors definitely are more conspicuous in salted butter than in unsalted butter made from the same lot of cream.

The flavors in the cream as it goes to the churn are determined by the flavors in the cream coming to the plant and by the methods used in the plant. The causes of undesirable flavors in the original cream are the same as for milk; for sweet cream the feed and condition of the animal are of the greatest significance, and for sour cream the action of organisms is the most important. The principal defects in the plant methods that influence the flavor of butter are improper neutralization or pasteurization, and culture of poor quality.

Causes of undesirable flavors that develop in butter. Although the flavor of butter may improve for a short period after its manufacture through the decrease of some objectionable condition, such as a heated flavor, or through intensifying of the desirable flavor, the general effect of holding is a decrease in quality. Extent of the decrease varies greatly; in butter properly made from high-quality cream and stored at suitable temperatures it may be negligible, but in a poorly made product stored under unfavorable conditions it may be very serious.

Changes in butter that result in development of undesirable flavors involve bacterial, enzymatic, and chemical action. Those which are in themselves strictly chemical may be greatly influenced by products of the growth of organisms, especially acids, so that organisms play a role in butter deterioration, both directly and indirectly.

In considering the flavors that develop in butter during holding, the ability of the product to absorb odors must be recognized. Because of this absorbing power, butter should be kept away from materials having pronounced odors. Butter of excellent quality often acquires an objectionable flavor by being held in an ice box containing other food products; such an exposure is especially serious after the package is opened. Under plant conditions the odor of a refrigerator in which butter is held is of importance in this connection, especially when the product is exposed in boxes or trays.

FACTORS INVOLVED IN BUTTER DETERIORATION

Differentiation of bacterial, enzymatic, and chemical changes in butter deterioration sometimes is difficult. Temperatures and general conditions that favor development of bacteria also may accelerate action of enzymes, and some of them favor certain chemical changes. In attempting to correct a specific flavor defect in butter, the first step is to determine its cause, that is, whether it is bacterial, enzymatic, or chemical. Often this is evident from the nature of the defect and corrective measures are easily formulated, but in other cases there is more than one possibility and the problem requires investigation.

The study of butter deterioration is complicated by the necessity of basing conclusions as to the extent of the change on the senses of taste and smell, rather than on definite measurements. It is difficult to keep exactly the same standard in judging a lot of butter on different days and the extent of the deterioration may be incorrectly rated.

Bacterial action. The early studies on the bacteriology of butter were suggested by the frequency with which the product deteriorated, and by the importance of organisms in bringing about changes in milk and cream. Pasteurization was introduced into the butter industry to control deterioration in the product after its value in preventing bacterial changes in various materials had been established. Unsalted butter made from raw cream normally undergoes bacterial deterioration when held at temperatures permitting growth of organisms; various defects develop, especially cheesiness and rancidity. Appearance of the defects can be delayed or prevented by limiting growth of the causative organisms through salting or the presence of considerable acid in the butter, but these procedures often are objectionable from the standpoint of chemical changes in the product. At present, proper pasteurization of the cream is recognized as one of the important factors in manufacturing butter that will keep satisfactorily when held at temperatures prevailing during distribution and consumption of butter. Development of the butter industry to its present importance would have been impossible without pasteurization, or some comparable process, for treatment of the cream. When butter made from pasteurized cream, with or without salt, shows bacterial deterioration, the causative organisms normally are present in the product because of inefficient pasteurization or contamination subsequent to heating.

At one time there was a definite tendency to ascribe all butter deterioration to action of bacteria. Evidence gradually accumulated which suggested that butter often deteriorates under such conditions that bacterial action is improbable. Experimental results also showed that butter could deteriorate at fairly high temperatures without an active increase in organisms; for example, Rogers [73] noted that canned butter stored at room temperature developed off flavors during a period when the numbers of organisms, as determined by both aerobic and anaerobic lactose gelatin plates, were decreasing.

Data from various sources indicate that when the butter is held at low temperatures addition of organisms to the cream from which it is made has little influence on the keeping qualities. Rogers *et al.*[74] compared butter from (1) raw cream, (2) pasteurized cream, and (3) pasteurized cream inoculated and held so that after 24 hr the numbers and types of the organisms approximated those before pasteurization without an appreciable increase in acidity having occurred. The reinoculation of the cream had little or no effect on the keeping qualities. Grimes [39] added certain proteolytic bacteria, various yeasts, and *O. lactis* individually to both ripened and unripened cream without the keeping qualities of the butter being significantly influenced during storage for 6 mo at −21.1 C (−6 F); mixtures of *O. lactis* and various yeasts gave the same general result.

Enzymatic action. Cream to be made into butter contains the enzymes that normally are found in milk and also the extracellular enzymes that may have been produced by organisms growing in it, or in the milk from which it came. Some of these enzymes bring about the types of changes that are important in butter deterioration and they may be of importance under certain conditions. Pasteurization destroys some of the enzymes and decreases the activity of others. Temperature has a pronounced influence on enzymatic action generally, so that enzymes might be of importance at one temperature and not at another. Sodium chloride in the concentration present in butter also may have an effect.

Rogers [73] reported data indicating the activity of lipase in butter. Comparing the increase in acidity in unheated and heated butter, he found that in the unheated material there was considerable increase in acid but in the heated material, in which the enzyme presumably had been destroyed, the change in acidity was negligible. Thatcher and Dahlberg [75] studied six samples of butter and found the lipase activity during 4 days at 40 C (104 F) too small to be accurately measured, except with one sample of stored butter. Under practical conditions rancidity has been noted in raw-cream butter when it appeared lipolytic organisms were not responsible.

Rogers *et al.*[74] found that in raw-cream buttermilk to which chloroform had been added activity of protease was almost entirely inhibited by the presence of 18 per cent sodium chloride and a temperature of −17.8 C (0 F); when some of the same material was allowed to stand for a period at room temperature, the protease apparently became more active because the casein clotted and the mixture assumed an appearance suggesting that digestion had occurred. A trial with a large quantity of a bacterial enzyme preparation obtained from a proteolytic, acid-forming organism indicated that milk proteins were hydrolyzed at −6.7 C (20 F) in the presence of a high sodium chloride concentration. Pancreatin and pepsin also gave proteolysis under these conditions. Sodium chloride may have had an inhibiting effect in the trials carried out. Combs and Eckles[76] concluded from their studies that the abnormal flavors which developed in butter as a result of mold growth on the cream were caused by enzymes secreted by the mold. *O. lactis* and a *Penicillium* were used alone and in combination. Pasteurization at 62.8 C (145 F) for 20 min or 73.9 C (165 F) for 5 min greatly improved the keeping qualities of butter made from cream on which molds had grown, but did not entirely check the action of the enzymes which had been produced. Thatcher and Dahlberg[75] reported that the normal milk enzymes need not be considered a factor in the deterioration of butter during storage because of the small amounts in which they are present and the unfavorable influence of storage temperatures on enzyme activity; these investigators found that sodium chloride inhibits protease to such an extent that the enzyme cannot act in normal salted butter.

In general, it appears that enzymes are of relatively little importance in pasteurized-cream butter but that certain enzymes, especially lipase, may be of significance in raw-cream butter.

Chemical action. Inadequacy of the action of organisms as an explanation of butter deterioration under all conditions gradually led to the conclusion that chemical changes are important. Such changes undoubtedly occur at the various temperatures to which butter is subjected; under storage conditions they may be the principal cause of deterioration, and at higher temperatures they may be relatively less important because of the influence of other factors, although actually more extensive. The chemical changes are of various types and a number of factors have very definite effects on them.

Influence of air. Relationship of air to butter deterioration has long been suspected because of the frequency with which the surface portion of butter shows a more undesirable flavor than the inner portion. Air tends to favor growth of certain organisms in butter and may influence deterioration in this way, but such an effect would not be operative at very low temperatures and chemical action is indicated. Gray [77] found that under various conditions of storage, including storage at −23.3 C (−10 F), butter in full cans kept much better than in cans partially full, and ascribed this to the presence of air in the unfilled cans. Dyer [78] studied oxidation in butter by analyzing the gas present after varying periods of holding; trials were carried out with butter made in various ways and kept at different temperatures. He concluded that the production of off flavors so commonly met with in cold-storage butter is attributable to a chemical change, expressed through slow oxidation that is progressive in some one or more of the non-fatty constituents. Extent of the change was proportional to the quantity of acid present in the cream from which the butter was made; at −17.8 C (0 F) it was slight in butter made from sweet cream and held 180 days, only a little greater in butter made from cream containing 0.25 per cent acid as a result of addition of 15 per cent butter culture and held for 205 days, but very pronounced in butter made from cream containing 0.71 per cent acid through addition of commercial lactic acid and held 202 days. With fat alone oxidation was slight; with buttermilk it was rapid when acid was present, but slight in the sweet product. The quantity of carbon dioxide in cold-storage butter appeared to have a certain relation to the quantity of buttermilk in the butter; during storage this quantity sometimes increased and then decreased.

Guthrie [79] noted that one effect of working butter is to increase slightly the percentage of incorporated air. The average for butter worked fairly well was 4.651 per cent and for butter thoroughly worked 5.374 per cent.

Influence of acid. At one time it commonly was believed that development of acid in cream intended for churning gave the butter more satisfactory keeping qualities. Presumably, this idea resulted from the ease with which acid foods can be preserved and ignored the early experimental data. In 1889, Conn [80] questioned the superior keeping qualities of ripened-cream butter. Patrick [81] reported one comparison of sweet- and ripened-cream butter in which the former appeared to have slightly better keeping qualities. Patrick *et al.*[82,83] carried out trials and found that sweet-cream butter deteriorated less than that made from ripened cream. These early experiments are of special interest because of the comparatively high storage temperature, approximately 10 C (50 F), used in most of the trials. Gray[77] noted that butter made from cream that was sweet and in a good condition kept well when stored at either −23.3 C (−10 F) or −12.2 C (10 F) and also after removal from storage, whereas butter made from cream that was sour and in a fair condition kept well at these temperatures but deteriorated rapidly after removal from storage; culture was used with both types of cream. In a study of fishy butter Rogers[84] found that in all cases where the records were complete the experimental butter that became fishy was made from high-acid cream, and that fishiness developed in butter made from cream acidified with lactic or acetic acid; cream with a high acidity did not uniformly develop fishiness, however. He stated that in his opinion fishy flavor is caused by a slow spontaneous chemical change to which acid is essential and which is favored by the presence of small amounts of oxygen. Rogers and Gray[85] concluded that butter frequently undergoes marked changes, even when stored at very low temperatures, and that these changes are more marked as the acidity of the cream from which the butter is made is increased; changes in the high-acid butter were not checked by heating the ripened cream. Rogers *et al.*[86] compared the deterioration in butter made from unpasteurized ripened cream with that made from pasteurized sweet cream, the two types of butter being made in different creameries. The former had an average score of 94.20 when fresh and 87.25 after 8 mo in storage; the latter had an average score of 94.69 when fresh and 92.33 after storage. Fishiness was very common in the unpasteurized ripened-cream butter, whereas it was rare in the pasteurized sweet-cream product. Additional comparisons by these investigators gave similar results.

Butter made from sweet cream lacks the flavor given by the culture organisms and attempts have been made to obtain both the desired flavor and good keeping qualities by use of low acidities in the cream. Mortensen[87] reported data on butter made from (1) sweet cream, (2) sweet cream plus culture, and (3) ripened cream. In general, fresh ripened-cream butter scored higher than the other types but deteriorated more rapidly; at the end of 9 mo in storage the score was lower. Sweet-cream butter scored lower than the other types when fresh but, because of the slow deterioration, scored higher after long holding. Butter made from sweet cream with culture added kept better than ripened-cream butter and, both when fresh and after holding 2 mo, scored higher than sweet-cream butter. White[88] reported results showing the deterioration of butter made from sweet cream and from cream with various acidities, the lower acidities being obtained by addition of culture, whereas for the higher acidities the cream was ripened. All the cream was pasteurized at 62.8 C (145 F) for 30 min; in some cases pasteurization was carried out after adding the culture, but this did not appear to have any material influence on deterioration of the butter. The following table, representing 125 churnings, gives the deterioration of the butter during storage at −17.8 C (0 F) for 8 mo.

INFLUENCE OF ACIDITY OF THE CREAM ON DETERIORATION OF BUTTER

Acidity of Cream When Churned, Percentage	Score of Butter		Decrease in Score during Storage
	When Fresh	*After Storage*	
0.15	91.02	90.17	0.85
0.25	91.09	90.14	0.95
0.31	91.02	89.83	1.19
0.35	91.23	88.73	2.50
0.45	90.94	87.39	3.55

The results show very little deterioration in the butter made from cream having 0.15 or 0.25 per cent acid; when the cream had 0.31 per cent acid deterioration was slightly greater and, when it had 0.35 per cent, or more, acid deterioration was pronounced. The data suggest that, when the butter is to be stored for 8 mo at low temperatures, acidities up to 0.31 per cent give comparatively little deterioration; other results presented by White indicate that if the storage is under less favorable conditions there may be considerable deterioration with an acidity as high as this.

Studies involving *p*H determinations on butter serum also indicate the influence of acid on chemical changes in butter. Arup and Gilmour [57] noted that butter with *p*H values below 6.7 did not keep so well in storage as butter with values above this. Gilmour [89] reported data on 1,292 samples of butter from 185 plants over a period of 4 mo. The *p*H values ranged from 5.70 to 7.70; there was a decided tendency for the flavor score to diminish as the *p*H decreased. Gilmour and Arup [90] found that *p*H determinations are a better guide to keeping qualities in butter than titration values. Butter with high *p*H values kept much better than that with low *p*H values; a tentative *p*H standard of not less than 6.70 was suggested for butter to be stored. White [91] noted that acidity of the cream and therefore *p*H of the butter serum had a definite effect on the flavor scores of butter. As the average acidity of the cream increased from 0.11 to 0.20 or 0.31 per cent the average *p*H of the butter serum decreased from 6.76 to 6.1 or 5.51, respectively, with a resulting loss in flavor scores of 2 to 3 points between the low-acid and the high-acid products. In medium- and high-acid butter, the principal flavor defects were metallic, sour, or acid, and in one case fishiness. The results indicate that acidity of neutralized sour cream should be 0.15 per cent, or lower, to give a *p*H in the butter serum of 6.7 to 7.2 for the highest flavor scores on fresh and stored butter.

Other experimental data and extensive commercial experience emphasize the importance of low *p*H values in the deterioration of butter. Although the *p*H ranges suggested as the most desirable differ somewhat, the danger in low values is generally recognized. The desired *p*H in butter serum is obtained by neutralization of the cream and with good procedures the *p*H can be controlled within narrow limits. Various butter organizations regularly check the *p*H values of butter to be certain that the neutralization is being properly carried out. In addition to controlling the *p*H, neutralization may fix as salts certain undesirable acids in the cream and in this condition they are less important as a cause of objectionable flavors in the butter. In combination with proper pasteurization, neutralization makes possible the manufacture of butter with good keeping qualities from cream in which there has been extended action of micro-organisms; pasteurization controls the deterioration due to the growth of organisms, whereas neutralization limits certain types of chemical deterioration.

Acid influences the deterioration of butter primarily through its effect in increasing the rate of chemical changes. It is evident that other factors are involved, however, because certain lots of butter with low *p*H values keep satisfactorily. It is probable that the additional contributing factors vary from one lot of butter to another. Since the acid present in cream is due to activity of bacteria, its effect on the deterioration of butter is an indirect result of bacterial development, and in the final analysis the influence of acid is a bacteriological problem.

Influence of metal. Results of various investigations indicate the relationship of absorbed metals to butter deterioration. Rogers *et al.*[74] found that addition of iron to cream, even in as small amounts as 1 or 2 ppm, had an influence on the flavor of the butter; there was no evidence that the nature of the flavor was appreciably changed but rate of development was accelerated. Action of copper was similar but perhaps more intense. In general, only a relatively small part of the iron goes into butter as compared with buttermilk and, accordingly, a flavor due to iron was most noticeable in buttermilk. A large percentage of the iron retained by the butter was present in the curd. The investigators also found that a strong odor could be produced in milk by addition of small amounts of iron salts, the ferrous salts being more active than the ferric. Hunziker and Hosman [92] reported that direct contact of butter with either iron or copper developed a tallowy flavor and turned the butter white; the action began where the butter was exposed to air and gradually went through the entire mass. Copper apparently caused a more rapid and more intense action than iron. In trials carried out by Palmer and Combs [93] tallowiness was produced in butter by addition of 0.017 per cent copper lactate to either raw cream or cream that had been pasteurized at 79 to 80 C (174.2 to 176 F) for 15 min. In each of three experiments, typical tallowiness and bleaching occurred in the raw-cream butter before it appeared in the pasteurized-cream product.

More recent investigations have also emphasized the influence of metals in bringing about deterioration in butter, and copper has rather regularly been more active than iron. Loftus-Hills *et al.*[58] reported that butter acidity and copper content appeared to be the most important factors controlling keeping qualities in 70 boxes of salted butter from Australia. Hussong and Hammer [94] encountered instances in which bleaching and tallowiness in butter were accompanied by high copper contents that apparently were related to them; in one instance the high copper content in the butter came from copper-contaminated wrappers. Experiments in which copper, usually cupric chloride, was added to the cream showed the relationship of added copper to tallowiness and bleaching in butter.

The conspicuous defect caused by an excessive amount of copper in butter commonly is tallowiness, which may be followed by bleaching; these defects eventually appear in all butter so that actually the copper simply speeds their development. In certain instances copper has caused fishiness, particularly with a low *p*H in the butter. Presumably, when the effect of the copper has been less pronounced, the off flavor produced has not been extensive enough to be definitely characterized. The influence of copper in producing off flavors in butter is in agreement with its effect on the flavor of milk.

During the handling of milk and cream there ordinarily is an opportunity for the acquisition of small amounts of metal from the utensils and equipment. This may occur through mechanical removal and also through formation of various compounds. The amount of metal picked up by milk and cream is influenced by various factors, such as condition of the utensils and equipment, washing procedures used with them, reaction, temperature and period of exposure of the product, and exposure to air. The copper in freshly drawn milk may vary in amount, owing to feed and possibly other factors. However, such copper apparently is much less serious from the standpoint of butter deterioration than copper added during handling of the milk or cream.

Influence of general composition. Although it is improbable that the variations encountered in moisture and curd contents of butter under commercial conditions have a significant influence on the rate of deterioration, the presence of these constituents in butter results in a decrease in the keeping qualities, as compared to a more highly purified fat. The ghee made in various countries by heating unsalted butter and then straining it is a product that keeps well under severe temperature conditions; the heating very largely evaporates the water and the straining removes a part of the curd which the heat has tended to flocculate. Patil and Hammer [95] found that both ghee and butter fat kept much better than ghee with added water or than butter. Addition of water to ghee accelerated deterioration more than addition of protein.

Moisture and curd in butter evidently favor action of bacteria in the deterioration of this product, and it is probable that the moisture also favors both chemical and enzymatic action. The curd provides a source of objectionable decomposition products.

Evidence which is rapidly accumulating indicates that heat treatment of fat, particularly under certain conditions, tends to delay oxidation of the fat and thus may have a conspicuous effect on the keeping qualities of various dairy products containing fat.

Effect of carbonation. In some instances, butter has been churned and worked in an atmosphere high in carbon dioxide with the object of destroying objectionable bacteria and improving the qualities of the product, both when fresh and after storage. Hunziker [96] concluded that carbonating cream cannot be relied on to make such cream, or the butter churned from it, safe for human consumption through destruction of injurious bacteria, or to destroy organisms that are harmful to the flavor and keeping qualities of the butter; he also reported that carbonating does not improve the flavor or market value of butter and that carbonated butter keeps no better than uncarbonated but develops the usual bacterial defects if made from unpasteurized cream.

Prucha *et al* [97] found that carbonation of sweet cream tended to suppress certain types of bacteria but did not hinder others. The treatment caused a delay in the souring of the cream, the length of which depended on the temperature of holding and probably also on the extent and type of the contamination; at room temperature the delay was about 2 hr but at 1.7 C (35 F) several days were involved. When carbon dioxide was applied at churning time by charging the cream in the churn and replacing the air above the cream with the gas, no significant benefit was noted. The gas caused the fresh butter to taste sour and this flavor tended to cover up the flavor of the neutralizer used. Experiments under both laboratory and commercial conditions showed that carbon dioxide did not measurably affect the germ life in the butter or improve the flavor or keeping qualities. Storage of butter in the gas in an air-tight container completely inhibited molds and improved the keeping qualities at low temperatures but undesirable flavors eventually developed.

Experiments of Sherwood and Martin[98] indicated that carbon dioxide did not improve the qualities of butter but tended to lower the score. The gas had no effect on the bacteria in the butter. The rate of oxidation was as great in the carbonated as in the uncarbonated product.

Valley and Rettger[99] found that the treatment of raw milk with carbon dioxide at atmospheric pressure resulted in little or no inhibition or destruction of bacteria. In pure culture studies with various organisms there was a parallelism between bacteriostatic or bactericidal action and *p*H, and it appeared that any harmful effect of carbon dioxide on bacteria was due to decreased *p*H. The investigators concluded that carbon dioxide is necessary for the growth and development of the bacterial cell.

GENERAL TYPES OF CHANGES THAT OCCUR DURING BUTTER DETERIORATION

Although off flavors in butter may result from changes so slight that their detection chemically is impossible with the present methods, certain types of transformations are to be expected from the general nature of the constituents and these suggest some of the possibilities in butter deterioration. Chemical evidence obtained in the case of extensive deterioration in butter has confirmed the expected changes in this product.

Protein. Protein of butter may be broken down to compounds of varying degrees of complexity. When extensive enough the decomposition can be detected by determination of the water-soluble nitrogenous compounds, or of definite cleavage products, such as amino acids or ammonia. The objectionable odors of certain compounds commonly formed in protein decomposition suggest that their elaboration in butter would be responsible for undesirable conditions.

Lactose. Lactose is very susceptible to bacterial action, acid being the important product formed. Since various organisms can attack lactose, transformation of this constituent of butter is to be expected in certain instances. Pronounced changes in the lactose content can be detected by actual determinations. An increase in the acidity of butter may result from decomposition of constituents other than lactose and so cannot be interpreted as due entirely to transformation of the sugar. The established relationship between acid in cream and deterioration in the butter churned from it indicates that the formation of acid from the lactose in butter would be undesirable because of its indirect as well as its direct effect.

Fat. Changes in the fat of butter are of two types: hydrolysis and oxidation. Hydrolysis results in the formation of free fatty acids and glycerol but with either of these products there may be further changes. Attempts sometimes are made to detect hydrolysis by determinations of the acid number but the possibility of acid being produced from other constituents must always be taken into account. In some instances a definite odor suggestive of butyric acid is interpreted as indicating hydrolysis. Oxidation of fat may result in tallowiness, presumably through the effect on the oleic acid radical; this effect may involve changes other than actual oxidation.

SPECIFIC FLAVOR DEFECTS OF BUTTER

The specific flavor defects of butter can be divided into three groups: (1) those due primarily to off flavors in the cream, (2) those due primarily to unsatisfactory methods in the plant, and (3) those that develop primarily during the holding of butter. This division is not clear cut and a certain flavor sometimes appears under conditions that would class it with a group other than the one to which it ordinarily belongs; for example, a fishy flavor most often develops when butter is held but occasionally is present in freshly made butter. The grouping is useful, however, in that it emphasizes the important causes of off flavors in butter.

DEFECTS DUE PRIMARILY TO OFF FLAVORS IN THE CREAM

Various off flavors in the cream, whether due to organisms or other causes, may be carried into the butter. Some of these are removed in part, or rather completely, by pasteurization, particularly vacuum pasteurization; others definitely are not susceptible to such removal.

Feed flavors. Various feed flavors in butter are related directly to similar flavors in the cream. The most serious of these flavors are due to onions, garlic, French weed, peppergrass, etc., but certain of the very useful feeds, especially silage and legumes, may affect the flavor of butter.

Barny flavor. A barny flavor in cream due to the absorption of odors in the stable often is carried into the butter. A somewhat similar condition in cream occasionally is due to the growth of organisms, especially certain of the *Aerobacter* species.

Malty flavor. A malty flavor produced in cream through growth of *S. lactis* var. *maltigenes* is readily transferred to the butter. Since cream may show a malty flavor before there has been a significant increase in acid, the condition is noted at plants receiving sweet cream as well as at plants receiving sour cream. In some instances the cream from a certain farm quite regularly shows this flavor, due presumably to a rather constant contamination with the responsible organism.

Old cream flavor. Certain lots of butter show a flavor that is difficult to describe but suggests that the cream used was of poor quality because of the growth of organisms. It frequently is designated old cream flavor and definitely is due to growth of various types of organisms in the cream.

Yeasty flavor. A yeasty flavor in butter is due primarily to the development of the lactose-fermenting yeasts, *T. cremoris* and *T. sphaerica,* in the cream before it reaches the plant, although careless holding of the cream in the plant sometimes results in a yeasty flavor, or intensifies it if already present. Conditions making possible the growth of *T. cremoris* and *T. sphaerica* also result in rapid growth of the lactic acid bacteria so that the typical yeasty flavor is noted only in sour cream.

Musty flavor. Cream may have a musty flavor as a result of storage in a cellar or cooling tank having a musty odor, or molds may grow on cream subjected to favorable growth temperatures for extended periods; under these conditions the butter often has a musty flavor. Butter also may absorb a musty odor from churns that are not properly treated. In some instances molds are actively growing in the culture used, but such growth quickly is detected by the experienced culture judge.

Cheesy flavor. One of the most objectionable flavors in butter due to the condition of the cream is a cheesy flavor. Presumably, various organisms can produce cheesiness in cream if they grow in it for considerable periods, but extensive growth of lactobacilli is especially important in this connection. A cheesy flavor due to the condition of the cream should definitely be distinguished from cheesy flavors that result from the development of organisms in butter.

Metallic flavor. A very characteristic condition that is referred to as metallic sometimes is present in butter and can be detected by either taste or odor. It also is common in buttermilk. The flavor suggests the astringent effect given by certain metals when they are taken into the mouth.

A metallic flavor in butter is due primarily to the same condition in the cream at the time of churning. The flavor may be present in cream as it comes from the producing farms, or it may result from growth of organisms during holding in the plant. A metallic flavor in cream often disappears when the cream is held under conditions very favorable for growth of organisms. Because of its relationship to the condition of the cream, a metallic flavor in butter usually is present as soon as the butter is made. In buttermilk, however, the flavor readily develops if the product is held at temperatures permitting active development of bacteria.

Guthrie[100] studied the development of a metallic flavor in dairy products and concluded that the direct absorption of metals or the action of bacteria may be responsible. He reported that the organism involved is a strain of the *S. lactis* group. High acidity was a necessary condition for the development. The investigator pointed out that, except in the case of buttermilk, a high fat content in the medium is essential for development of a metallic flavor. He found that the condition may develop in cream of either good or poor flavor.

Baker and Hammer[101] investigated production of a metallic flavor in buttermilk and found that it may occur when the buttermilk is held in a glass container so that absorption of metal is improbable. A metallic flavor was produced in sterile buttermilk by inoculation of lactic acid culture or a mixture of *S. lactis* and either *S. citrovorus* or *S. paracitrovorus*. Uninoculated buttermilk, or sterile buttermilk to which culture had been added, became metallic more frequently at 12 C (53.6 F) than at room temperature. The results indicate that the normal culture organisms are capable of causing metallic flavor when their development is excessive.

It is probable that the flavor referred to as metallic can be due to more than one cause. Absorption of traces of metals from cans and equipment of various sorts, especially when such easily attacked metals as iron and copper are exposed, presumably is a factor in certain instances. Excessive bacterial action of the normal culture organisms also may be responsible. Because of the unfavorable conditions in butter for the development of most organisms, this growth ordinarily takes place in the cream. In many instances a metallic flavor has been noted in butter churned from cream ripened to a high acidity.

A metallic flavor in butter usually is soon replaced by some other flavor and is uncommon in storage butter. Fishiness often follows it and, since excessive acid in the cream is related to fishiness, the importance of acid in causing metallic flavor is suggested.

Flavors distinctly foreign to butter. Butter occasionally shows a flavor distinctly foreign to it, such as kerosene, gasoline, fly spray, or disinfectant. This usually is due to absorption of odors by the cream, but there may be an actual addition of some foreign material through carelessness. Absorption by butter may occur but is less probable than absorption by the cream on the producing farms.

DEFECTS DUE PRIMARILY TO UNSATISFACTORY PLANT METHODS

Cooked flavor. Fresh butter made from pasteurized cream commonly has a slight cooked flavor but this largely disappears within a few days. In some instances, however, the cooked flavor is pronounced and persists during the holding period so that it definitely is objectionable.

An excessive pasteurization exposure for all or a part of the cream is the primary cause of the cooked flavor. With an excessive temperature at the heating surface there is a tendency for the cream in direct contact with it to be overheated; when cream adheres to the heating surface and is subjected to high temperatures for extended periods, the cooked flavor is especially pronounced. Lactic acid culture may show a cooked flavor but ordinarily is not responsible for the condition in butter in which it is used.

Neutralizer flavor. Butter made from neutralized cream often shows more or less of a neutralizer flavor. Its extent is related to the amount of neutralizer required to reduce the acidity to the desired point and also to the method of use. If the neutralizer is not properly and quickly distributed, if the neutralization is carried too far and the cream then pasteurized, or if the cream is heated to a comparatively high temperature before the neutralizer has reacted with the acid present, an undesirable flavor effect is to be expected. There is no close relationship between the *p*H of the serum and a neutralizer flavor in butter. Brown and Parfitt [102] reported that the *p*H values of 892 samples of centralizer butter criticized as having a slight or distinct neutralizer flavor ranged from 4.15 to 7.96.

Flat flavor. Butter made with culture so that it should show a full flavor sometimes does not and is referred to as flat. In general, this is the result of culture of poor quality, although in some cases a desirable flavor disappears from butter as a result of a destruction of the biacetyl originally present. Biacetyl and also acetylmethylcarbinol can be destroyed by various organisms.

High-acid or sour flavor. A flavor suggestive of considerable acid sometimes is noted in butter. Commonly, it is the result of excessive acidity in the cream at churning and this may be due to the condition of either the cream or the culture. When a culture simply forms acid instead of giving a typical flavor, there is a tendency for the butter in which it is used to show the defect. Retention of excessive amounts of buttermilk in the butter also tends to cause the condition.

DEFECTS THAT DEVELOP PRIMARILY DURING THE HOLDING OF BUTTER

Storage flavor. Butter that has been held in storage for a considerable period commonly shows a flavor that the experienced butter grader recognizes as storage flavor. The intensity varies greatly, and with properly made butter is slight. The condition probably is due to slow chemical changes; although such changes are reduced to a minimum by high-quality raw material and proper manufacturing methods, they cannot be entirely prevented and some deterioration must be expected when butter is stored for an extended period.

Rancidity. A flavor suggestive of butyric and caproic acids sometimes develops in butter and is described as rancid. The acids are set free through hydrolysis of fat. Rancidity in butter shows great variations and may be so modified by other off flavors that its detection is difficult. In some cases the products of hydrolysis appear to be changed, presumably through action of organisms or chemical reactions with constituents of the butter. Various products other than butter may develop rancidity.

Micro-organisms constitute an important cause of the hydrolysis in butter that results in rancidity. In the early studies of Orla-Jensen [103] a number of species were found which produced rancidity, including *O. lactis, Cladosporium butyri, Ps. fluorescens,* and *Ser. marcescens.* Other organisms also can produce the change. *Ps. fragi* has been repeatedly detected in rancid butter in numbers sufficiently large to indicate its causal relationship to the defect. Hammer and Collins [61] found lipolytic bacteria common in raw milk and cream. Rancidity often developed in butter churned from raw cream and held at 6 C (42.8 F); frequently, this butter contained large numbers of lipolytic bacteria. Butter made in a carelessly treated churn from pasteurized cream commonly developed rancidity when stored for a long period at 0 C (32 F). There is some evidence that the lower fatty acids, such as butyric and caproic, are rather toxic for organisms and this may account for the relatively small numbers of organisms in certain lots of rancid butter.

Lipase may be a factor in development of rancidity in butter made from raw cream. In some instances butter made in commercial plants from raw cream, because of failure of the pasteurization equipment, has developed rancidity without showing significant numbers of lipolytic organisms on plating. A rancid flavor is common in butter made on farms from unpasteurized cream; apparently, lipase may be involved as well as lipolytic micro-organisms.

Jack *et al.*[104] separated cream from milk that developed rancidity, and also from normal milk, and churned it. Provided the milk was pasteurized at 65.6 C (150 F) for 30 min, at 76.7 C (170 F) for 20 min, or at 82.2 C (180 F) flash within 18 hr after it was drawn, the butter made from either type of cream and stored at −6.7 to −5.6 C (20 to 22 F) did not deteriorate in quality up to 60 to 65 days, as shown by flavor score of the butter and acidity of the fat. When either type of cream was held raw at 4.4 C (40 F) for 3 days before pasteurization, lipolysis occurred to the detriment of the resulting butter; butter from milk that developed rancidity was inferior to that from the normal milk. Rancidity was not recognized organoleptically until the acid degree approached a value of 2.0.

Organisms and lipase are the primary causes of rancidity in butter. Guthrie[105] noted that exposure of butter and butter fat to high temperatures, light, and air did not cause rancidity. Fatty acids giving a rancid odor can be liberated from butter fat by strong acids, but such a change probably never occurs under practical conditions.

In general, the organisms that cause rancidity in butter are easily destroyed by heat and, since lipase also is easily inactivated, proper pasteurization and protection against recontamination make it possible to produce butter that will not become rancid. In modern butter plants rancidity is one of the defects of butter that is completely controlled.

Fishiness. Development of a fishy flavor in butter is one of the very serious types of deterioration. It is primarily a defect of butter that has been stored, but occasionally appears in fresh butter when the acidity at churning is relatively high. Some of the heavy financial losses in storage butter have been due to development of pronounced fishiness in butter that reached storage in an excellent condition.

The early investigations of fishiness led to various conclusions. O'Callaghan[106] cited experiments which suggested that *O. lactis* is the cause of the defect. Later,[107] he reported that when this organism was grown in conjunction with *S. lactis* fishiness was produced on every occasion; he also noted that many consignments of unsalted Australian butter were fishy in London and that fishiness sometimes is detected in unsalted butter before it leaves Australia. In the United States fishiness in unsalted butter is extremely rare. The idea that salt may cause fishiness was considered by Piffard,[108] who pointed out the ability of salt to absorb the odor of material stored near it. He also stated that fishy flavor in milk sometimes is the result of the flavor in water consumed by the cows, this being due to the development of diatoms and algae.

Rogers[84] concluded from his study of fishy butter that the flavor evidently is not produced by the action of any one special factor, and that results indicate its immediate cause is a substance produced through oxidation of one of the combinations of the acid developed in the ripening of cream. He noted that acid is essential and that butter made from pasteurized sweet cream with a culture, but without ripening, seldom if ever becomes fishy; overworking the butter made from sour cream produced a fishy flavor with reasonable certainty. The investigator was unable to produce fishy butter by inoculation of *O. lactis* into the cream, and in samples of fishy butter examined only very small numbers of the species were found.

Reakes *et al.*[109] were unable to find significant differences in the bacteria of fishy and high-grade butter. Plugs of fishy butter put into high-grade butter failed to cause fishiness, and plugs of high-grade butter placed in tubs of fishy butter retained their desirable flavor. With the present information on the multiplication of organisms in butter, migration of bacteria from one portion of butter to another would not be expected. The investigators concur in the idea that a chemical change results in a splitting of some butter constituent into compounds having a fishy flavor.

The fishy odor of trimethylamine naturally suggested this compound as a cause of fishiness in butter. Although Rogers[84] was unable to produce fishiness by working large quantities of it into butter, Supplee[110] obtained a fishy flavor when trimethylamine was worked in under proper conditions, especially when trimethylamine butyrate was used. Supplee noted that normal butter contains lecithin, which can yield trimethylamine on decomposition; trimethylamine was found in some samples of fishy butter but not in others. Sommer and Smit [111] reported that addition of trimethylamine lactate to butter caused a distinct fishy flavor, and that trimethylamine could be isolated from samples of fishy butter; butter made from cream to which lecithin had been added became fishy more readily than butter made from untreated cream of the same lot. The investigators noted that organisms that produced trimethylamine from lecithin under favorable conditions failed entirely to grow in the presence of salt and acid in the concentrations found in the moisture of butter.

With chemical decomposition of lecithin the cause of fishiness, the factors influencing this process become of importance. Acid favors the hydrolysis of lecithin and may increase the content of metals that can act as catalysts. Salt solution is a good solvent for lecithin and thus may change the lecithin to such a condition that chemical decomposition can go on more rapidly; this explains why fishiness is primarily a defect of salted butter. Overworking favors the solution of the lecithin and may distribute the incorporated air more uniformly. Sommer and Smit [111] pointed out that pasteurization tends to eliminate fishiness because it causes some hydrolysis of the lecithin, the products of which are lost in the buttermilk, and because during the heating the lecithin absorbs oxygen and becomes less soluble.

Cusick [112] reported that fishy flavor was produced in butter by inoculating the cream with *Pr. ichthyosmius.* Age and acid content of the cream and period of incubation before churning appeared to influence production of the flavor in the butter.

In a trial carried out by Horrall [113] lecithin disappeared much more rapidly in salted butter made from high-acid cream than in salted butter made from sweet cream. However, none of the butter was criticized as fishy, which suggests that lecithin decomposition does not always yield trimethylamine.

Hussong *et al.*[114] found that fishy butter from various plants commonly had lower *p*H values than non-fishy butter from the same plants at about the same periods. Butter from one plant developed fishiness at higher *p*H values than that from the other plants, suggesting that a factor other than *p*H also was operative. In various instances in which fishy butter had a relatively high *p*H, it contained comparatively large amounts of copper. The authors noted that cream from certain producing areas apparently yields butter that is more susceptible to development of fishy flavor than cream from other areas; this may be related to the feeds consumed by the cows.

Although fishiness is much less common than formerly because of the relatively low churning acidities now used, it still is a serious defect. It is encountered in relatively fresh butter, and also in storage butter. With the latter product the intensity often greatly increases during the period the butter is in retail channels; this may be due partly to a temperature effect, but it appears that when the chemical change resulting in fishiness is once begun it continues at an accelerating rate. In the control of fishiness the first consideration should be the *p*H of the butter serum. Hussong *et al.*[114] noted that when cream comes from a variety of sources control of the *p*H may be difficult because of some unusual relationship between titration of the cream and *p*H of the butter serum. They studied a plant in which the butter began to develop a fishy flavor following receipt of considerable volumes of cheese-factory cream, both whey and whole-milk cream. The *p*H values of the butter were relatively low although the churning acidities had not been changed. Eventually, it was learned that certain cheese factories were skimming a very rich cream and then diluting with cold water to cool the cream and thin it sufficiently to make handling easier. Additiontion of the water changed the buffer capacity of the cream so that the *p*H at a given acidity was considerably lower than it otherwise would have been. If the *p*H of the butter is 6.8, or above, the possibility of excessive copper contamination should be considered. With cream from a variety of sources this also involves the possibility of some unusual situation, such as excessive copper contamination from poorly tinned cheese vats.

Cheesiness, surface taint, rabbito, etc. Butter shows a series of more or less closely related off flavors that range from a relatively slight abnormality through defects in which the odors suggest cheddar cheese, swiss cheese, limburger cheese, or a putrid condition. The defects commonly develop quickly. Often they appear first at the surface and are most conspicuous there, but eventually they are evident throughout the mass of butter. Various explanations have been suggested for the defects but action of bacteria in the butter is responsible; the general characters of the off flavors suggest that the primary effect is on the protein. *Ps. putrefaciens* appears to be the common causative species. When it develops in butter, the off flavor first is slight, then becomes more and more objectionable, and finally is less conspicuous. This series of changes explains the variations noted under practical conditions.

Eckles[115] reported an outbreak of putrid butter that occurred in 1900 and lasted about 2 weeks. The defect was so pronounced the local sale of butter was stopped and what had been sold was returned. The plant was in good condition but the milk received was of unsatisfactory quality. Changes involving rejection of poor-quality milk, better care of utensils, and use of lactic acid culture were instituted at the plant, and about the same time a long, dry period was broken by heavy rains. The butter improved at once and no further difficulty was experienced. The putrid butter contained a number of liquefying bacteria that included some forms that had a very injurious effect on butter. Eckles concluded that the putrid condition was brought about by contamination of the milk.

FIG. 37. Bacteria in putrid butter. Courtesy R. V. Hussong.

In Canada, a defect commonly described as surface taint has been encountered frequently. The defect begins at the surface, and at comparatively high temperatures may develop rapidly. According to Hood and White,[116] it made its appearance, in 1919, in first-grade pasteurized cream butter sent to the Vancouver market. The investigators noted that butter with this defect showed abnormally high yeast and bacterial counts, and that all the samples contained large numbers of bacteria capable of decomposing curd. They also found that mold, yeast, and bacterial counts on butter from plants having difficulty with surface taint usually were high, although with some factories variations in the results suggested inconsistent sanitary control. Many samples of raw-cream butter contained fewer bacteria than surface-taint butter made from pasteurized cream. Nothing abnormal was noted in the acid or curd content of butter showing the defect. The investigators produced surface taint in experimental butter made from pasteurized, neutralized cream by inoculating into the cream certain bacteria isolated from water; without inoculation, the defect did not develop.

Sutton [117] reported that two striking characteristics of a decomposed odor in butter are the rapidity with which it may develop in butter apparently of satisfactory quality, and lack of correlation of the condition with the numbers and types of bacteria which develop on beef extract agar.

Derby and Hammer [118] found that surface-taint butter often contained large numbers of bacteria although with a few samples the counts were comparatively low. Counts were higher on the surface portion of a sample than on the interior in all but 1 of 20 comparisons. Surface taint could not be produced in butter by inoculating a normal product, either salted or unsalted, with defective butter, but could be developed by inoculating the defective butter into pasteurized cream and churning the cream. Two to 4 days were required for the defect to develop at 15.6 C (60 F), and 7 to 10 days at 5 C (41 F). The defect sometimes developed in commercial butter held at temperatures very favorable for bacterial growth. In most cases the butter that developed surface taint was unsalted or had a low salt content. The organisms that predominated on beef infusion agar plates poured with surface-taint butter did not produce surface taint when inoculated into pasteurized cream which then was churned. A number of organisms that would produce surface taint were isolated from defective butter, but always were found in small numbers. An organism which regularly produced the defect in unsalted butter was first isolated from Canadian butter; it was designated *Achromobacter putrefaciens* but now is included in the genus *Pseudomonas.* Later, it was obtained from additional samples of surface-taint butter. The organism was greatly restrained by use of medium percentages of salt or by culture.

Cullity and Griffin [47] investigated outbreaks of rabbito in Australian butter and emphasized the importance of water as a source of the causative organisms. Apparently, *Ps. putrefaciens* was isolated from two supplies, although the authors indicated there were variations in the characters of the organisms.

Experience in various laboratories indicated that special isolation procedures were needed in studying cheesy, surface-taint, and rabbito butter. Claydon and Hammer [119] isolated *Ps. putrefaciens* from commercial putrid butter most easily by inoculating the butter into thoroughly pasteurized cream, churning the cream, and smearing the butter on agar after the defect had developed. Inoculation of the defective experimental butter into cream for production of second or third generation samples occasionally resulted in isolation of *Ps. putrefaciens* when it had not been obtained previously. Incubation of the plates at 5 to 10 C (41 to 50 F) also aided in the isolation. The investigators noted that the organism did not readily initiate growth on media. With the isolation procedures used, they obtained it from 41 (70.7%) of 58 samples of typical putrid butter, 6 samples (10.3%) yielded an apparent variant form, and 3 samples (5.2%) yielded organisms producing objectionable odors not typical of the putrid defect. The *p*H values of the commercial putrid butter varied from 5.8 to 6.8.

For the isolation of *Ps. putrefaciens,* Linneboe [120] used nutrient agar at *p*H 7.2 to 7.5. and before pouring the plates added sterile ferric ammonium citrate at the rate of 0.5 ml of a 10 per cent solution per 10 ml of agar. The plates were incubated 7 days at 25 C (77 F). Any well-isolated colony that had made a depression in the medium varying in extent from slightly sunken to one resembling gelatin liquefaction was studied in detail for final identification. Linneboe found *Ps. putrefaciens* in a number of water supplies.

Long and Hammer [121] developed a special medium for isolation of *Ps. putrefaciens* that has the following composition:

	Per Cent
Gelatin	4.0
Proteose peptone	2.0
Dipotassium phosphate	0.1
Ferric ammonium citrate	0.05
Agar	1.5
Water to make	100.0

Smearing the plates was better than pouring in the usual way. The organism develops readily on the medium and after several days colonies are fairly large, raised, and brown to reddish brown or pink; however, growth may not occur when the numbers of organisms in the inoculating material are small. · Often *Ps. putrefaciens* colonies can be detected in an area heavily seeded with other species. In isolations from butter, the serum was more satisfactory than the butter direct. With various materials enrichment in litmus milk at 3 C (37.4 F) frequently was helpful. *Ps. putrefaciens* was found widely distributed in certain dairy products, in water, on dairy plant floors, in dairy plant sewers, and in dairy plant equipment. It also was isolated by direct smear and by enrichment from churns[35] and from insulation of a leaky vat; at certain points the organism was present in considerable numbers.

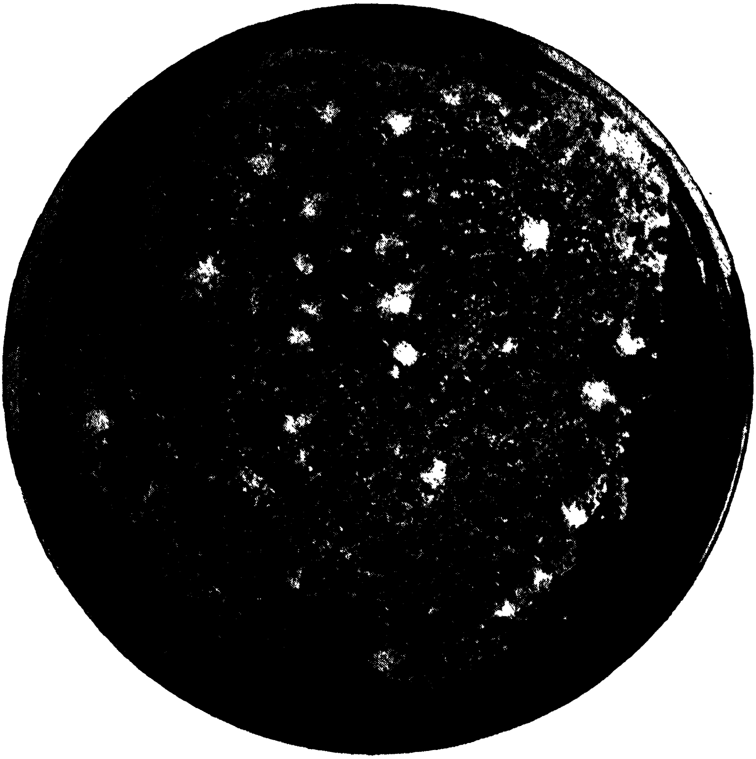

FIG. 38. Large colonies of *Pseudomonas putrefaciens* among smaller colonies of other species on special medium of Long and Hammer.

Pont[122] noted that the original source of infection of butter with rabbito organisms in the plant appears to be the water supply but that secondary, and in some cases more serious, foci of infection may be set up in churns and other equipment, especially when made of wood. Heavy salting, high acidities, thorough working, and efficient manufacture assist in minimizing or controlling the defect, but these factors cannot be relief upon for complete control in the presence of adequate infection and suitable temperatures for growth. Itzerott [123] reported that rabbito occurred most frequently in butter showing open texture and free moisture. He also noted that butter with a heavy infection of rabbito organisms showed no sign of the defect when stored for 3 mo but on removal from storage developed the defect, usually in 30 to 40 hr, at 18.3 to 35 C (65 to 95 F).

Wolochow *et al.*[124] considered that the chemical compounds imparting a sweaty-feet odor to skim milk cultures of *Ps. putrefaciens* are the same as those causing the odor in commercial surface-taint butter. Dunkley *et al.*[125] concluded that acid mixtures obtained by steam distillation of acidified skim milk cultures of *Ps. putrefaciens* contain formic, acetic, butyric, and iso-valeric acids, and that the substance causing the sweaty-feet odor of skim milk cultures of *Ps. putrefaciens* is iso-valeric acid. Wolochow *et al.*[126] reported that surface taint did not develop in butter churned from cream containing *Ps. putrefaciens* cells that had been treated with such odorless germicides as mercuric chloride; this indicates that living bacteria in the butter are necessary for development of the defect.

It would be expected that organisms other than *Ps. putrefaciens* could cause a putrid condition in butter. In agreement with this, Wolochow *et al.*[127] reported that a yellow pigmented organism, which they named *Flavobacterium maloloris,* produced a mild sweaty-feet odor in litmus milk and mild surface taint in butter churned from inoculated, highly pasteurized cream. They believed it less important than *Ps. putrefaciens* as a cause of commercial surface taint. These were the only two species suspected of surface-taint-producing ability among 2,500 random isolations from normal and abnormal butter and creamery well water. Reynolds and Thornton [128] encountered only two cultures of *Fl. maloloris* among 589 isolations of yellow-colony-producing bacteria from 140 samples of Alberta water. Studies on steam distillate residues of milk cultures of the organism indicated the same volatile acids as were reported for *Ps. putrefaciens.*

Long and Hammer [121] sealed various strains of *Ps. putrefaciens* grown in milk for 1 day and 7 days in glass tubes and exposed them in a water bath at 61.7 C (143 F); the cultures all survived for 0.5 min but most of them were killed in 1 min and the remainder in 2 min. When suspended in sterile distilled or pasteurized and filtered well water, *Ps. putrefaciens* [129] was readily destroyed by chlorine, provided excessive numbers of cells were not present; destruction was especially active in distilled water. Destruction was unsatisfactory when the numbers of organisms were excessive in relation to the chlorine concentration or its period of action. Among the strains of *Ps. putrefaciens* studied, there was evidence of some variation in chlorine resistance.

Elliker and Horrall [130] found that the first apparent stage of decomposition when *Ps. putrefaciens* developed in either commercial or laboratory butter was complete loss of typical butter aroma so that the butter became flat; later, cheesy or putrid flavors appeared. Chemical analyses indicated that during 7 days at 21.1 C (70 F) the biacetyl content of control butter samples remained constant but that more than one-half the biacetyl was destroyed in similar lots of butter contaminated with *Ps. putrefaciens.* The biacetyl content of butter prepared with culture and contaminated with *Ps. putrefaciens* remained relatively stable over a 7-day storage period at 21.1 C, although some destruction of biacetyl occurred. Elliker [131] reported that various bacteria, from widely differing genera, destroyed biacetyl added to sterile skim milk. Several *Pseudomonas* species isolated from butter and creamery water destroyed it in experimental butter. Tests indicated that *Ps. putrefaciens* reduces biacetyl to acetylmethylcarbinol and 2,3-butylene glycol.

Tallowiness. Butter sometimes develops a flavor suggestive of tallow. The defect varies from a very slight abnormality to an extreme condition in which the off flavor is pronounced and the butter may be bleached. Development of tallowiness in sterile butter fat indicates that the direct action of organisms is not involved.

According to the findings of various investigators, production of tallowiness in milk fat involves an oxidation, presumably of the oleic acid. In the extreme condition there is a definite decrease in the iodine number. Hunziker and Hosman [92] pointed out, however, that butter does not show a tallowy flavor when oleic acid, which has been exposed to the air until there has been a reduction in the iodine number, is added to it, but that butter containing free oleic acid rapidly becomes tallowy when exposed. Accordingly, these investigators consider that tallowiness is not the direct result of oxidized oleic acid, but is due to products formed in the butter during absorption of oxygen by oleic acid; since glycolic acid can be derived from oxidation of glycerol, esters of this compound are a possible cause of tallowiness. Trials showed that although addition of glycolic acid did not give butter a tallowy flavor, glycolic acid esters of the fatty acids of butter produced a tallowy flavor when warmed with butter fat, especially the glycolic acid ester of oleic acid. The tallowiness was produced in the absence of air and comparison samples, in which oleic acid alone was added, showed no tallowiness. The small amount of glycolic acid ester required to produce tallowiness is shown by the fact that 0.25 per cent of this reagent added to butter fat produced a distinctly tallowy flavor. The relationship of glycolic acid to tallowiness is suggested by the presence of glycolic acid in the fat of sheep's wool, which has a tallowy odor.

King[182] noted that in the presence of air butter fat reacted with biacetyl and became tallowy and bleached. The reaction was accelerated by light. King suggested that biacetyl may act on butter fat by oxidizing oleic acid to oleic acid peroxide that then breaks up into various compounds having a pronounced tallowy flavor, the biacetyl being reduced to acetylmethylcarbinol or 2,3-butylene glycol. In butter only a relatively small amount of the biacetyl is in the fat, the greater portion being in the serum, and the opportunity for reactions between the fat and the biacetyl is much less than when biacetyl is added to butter fat. Presumably, the mechanism suggested by King would apply to oxidations effected in various ways. The complexity of the problem from the standpoint of the chemical compounds formed is indicated by the many studies along this general line and the differences in the conclusions drawn.

Various investigators have emphasized the accelerating effect of traces of metal on development of the tallowy flavor in butter. Addition of a small amount of copper salt to cream at the time of churning quite regularly results in rapid development of tallowiness in the butter. This tallowiness occurs regardless of the *p*H of the butter serum.

Hussong and Hammer[94] reported various instances in which bleaching and tallowiness in butter were accompanied by high copper contents. In one instance there were blue areas on the butter before the bleaching began. Copper-contaminated wrappers, which had areas of brown discoloration, apparently caused a high copper content in one lot of butter studied.

When butter or butter fat is exposed to sunlight it quickly becomes tallowy and bleached. Diffuse light also accelerates production of tallowiness in these products. Under practical conditions, temperature is an important factor in the development of the defect. Butter held at 21.1 C (70 F) commonly develops tallowy flavor rapidly, and as the temperature of holding decreases the period required for production of tallowiness increases. Various lots of butter appear to differ in their susceptibility to the changes producing tallowiness, even when all the cream from which they were made was of fine quality. Tallowiness is a defect that regularly develops in butter if it is held sufficiently long, and lots of butter showing various serious abnormalities, such as fishiness or cheesiness, eventually become tallowy.

Wiley[133] found that in butter, low *p*H, presence of culture organisms, low pasteurization temperatures, and salt all separately favored oxidation of fat at cold storage temperatures. Oxidation in the presence of culture organisms, even when the acid formed by them was neutralized before churning, indicated some fat-oxidizing system in ripened cream. This could be some product of bacterial metabolism acting as a pro-oxidant, but it was not biacetyl or acetylmethylcarbinol. Wiley noted the good keeping quality of butter made from ripened cream which had been neutralized and then pasteurized and reported that such butter was no more markedly oxidized during cold storage than sweet-cream control butter. This suggests that if a pro-oxidant is formed during ripening it is destroyed by pasteurization. The general results indicated that the culture organisms contain a fat-oxidizing enzyme that is most active at low *p*H and high salt concentration; the effect was not peculiar to any particular culture species.

Preventing oxidation in butter is one of the most important problems in the butter industry. All butter eventually becomes tallowy; other conspicuous defects may or may not precede it. Tallowiness in a lot of butter continues to get worse over long periods. Apparently, no simple procedure prevents it. Butter made with or without culture becomes tallowy. Various antioxidants have been suggested for use in butter and sometimes may delay appearance of the defect but do not prevent it. There also may be some delay as a result of high heat treatment of the cream and this general procedure may prove to be especially useful. Elimination of copper contamination definitely is helpful in delaying the defect. The complexity of the chemical changes involved complicates solution of the problem.

Additional defects due to organisms. Chemical changes produce only a limited number of types of flavor defects in butter. Action of micro-organisms produces a variety of types; some of these are modifications of defects already described, whereas others are definitely different.

Organisms of the *Escherichia-Aerobacter* group sometimes produce unclean flavors in butter. The *Aerobacter* species are more important in this respect than the *Escherichia* species. Hammer and Yale[134] found that in butter both relatively low temperatures, such as 7 C (44.6 F), and salt have striking influences in restraining the organisms of this group. In 10 days at about 7 C, *Escherichia* species did not grow in salted butter, but in unsalted butter some of them did, whereas *Aerobacter* species sometimes grew in salted butter and regularly grew in unsalted butter. The *Escherichia* organisms did not develop off flavors in the butter; the *Aerobacter* organisms did not develop off flavors in salted butter but sometimes did in unsalted. In 10 days at about 18 C (64.4 F) both *Escherichia* and *Aerobacter* species grew in salted and unsalted butter, but the *Aerobacter* species grew more rapidly and reached higher numbers; the *Escherichia* species did not develop off flavors in the butter, whereas the *Aerobacter* species developed off flavors in either salted or unsalted butter.

An ester-like flavor sometimes appears in butter. Apparently, it can be produced by various organisms. When *Ps. fragi* is growing in butter an ester-like flavor often is noted before definite rancidity develops.

A skunk-like odor that developed in 3 samples of unsalted butter from one plant when they were held at 21 C (69.8 F) to test the keeping qualities was studied by Claydon and Hammer.[135] The defect was reproduced by churning pasteurized sweet cream inoculated with the defective butter. The causative organism was isolated and named *Pseudomonas mephitica.* On inoculating pasteurized cream used for churning, the defect developed in unsalted butter made from cream having *p*H values from 5.0 to 7.5. Salt had an inhibitory effect on production of the defect but thorough working was necessary to prevent its appearance. The skunk-like odor was produced in unsalted butter by inoculating small amounts of culture into the water used to wash the butter. Experience of various butter manufacturers indicates that a skunk-like odor sometimes is produced in butter as a result of the consumption of one or more pasture plants by the cows.

In certain lots of butter showing mold growth there is a flavor suggesting roquefort cheese. Presumably, *Penicillium roqueforti* and related forms are primarily involved. The probable cause of the flavor is the production of methyl ketones, especially methyl-*n*-amyl ketone.[136]

In rare instances it appears that a yeasty flavor has been produced in butter through the growth of lactose-fermenting yeasts.

With many species of organisms capable of growth in butter under favorable conditions, a variety of flavors would be expected. Many of them are inconspicuous and indefinite and are of relatively little commercial importance, although very evident to the butter grader. Often it is difficult to isolate the causative species and the defects actually may be due to associative action. The problem is complicated by the presence of organisms that may grow extensively in butter without any significant effect on the flavor. Occasionally, an unusual defect is encountered and often the causative organism can be isolated easily.

Sectional contamination. Jensen[187] reported on sectional contamination of butter in which localized areas take on a distinct off flavor so that there are variations in quality in different portions of the lot. The butter commonly shows zones of tainted product separated by zones of normal product. The defect does not occur in fresh butter but at 4.4 to 10 C (40 to 50 F), or higher, it may develop rapidly, sometime becoming distinct in 3 to 4 days, although as a rule development is more gradual. The off flavors encountered in the affected areas vary from staleness to distinct fruitiness, cheesiness, and rancidity. Observations indicated that the defective areas in a lot of butter showing sectional contamination tend to follow the junctions of the several pieces or layers of butter going to make up a block of hand-packed product. The defect was attributed to localized action of organisms carried to the surface of the butter by water and utensils used in packing operations. The primary source of the causal organisms was the water supply; a secondary source was the wood of the utensils, where the organisms survived washing and sterilizing procedures.

Control of microbiological defects. Complete control of the flavor defects of butter due to growth of micro-organisms requires attention to many details. In general, the species involved are destroyed by adequate pasteurization so their presence in the raw cream presents no problem. Following the pasteurization, contamination of the cream and of the butter must be avoided and this requires attention to the various sources from which organisms can come.

The restraining effects of physical condition, salt content, holding temperature, and acidity on growth of organisms in butter frequently prevents spoilage with methods of plant operation which would be expected to give it. This may lead to more and more carelessness in production procedures until eventually an outbreak of spoilage occurs. Because of the heavy contamination of wood churns, other equipment, and the surroundings, such an outbreak may be difficult to control immediately.

In some instances spoilage outbreaks develop in plants in which the operators believe they are using adequate precautions and then it is difficult to recognize possible sources of contamination. Under these conditions wood churns that, because of their age and mechanical state, are difficult to sterilize and contaminated water supplies appear to be at fault most frequently.

COLOR CHANGES IN BUTTER DUE TO ORGANISMS

Color changes occur in butter as a result of growth of organisms. Various molds are especially important in this connection, and occasionally pink yeasts or bacteria are involved. The color changes do not take place rapidly, but occur more readily in unsalted than in salted butter because of the restraining action of salt.

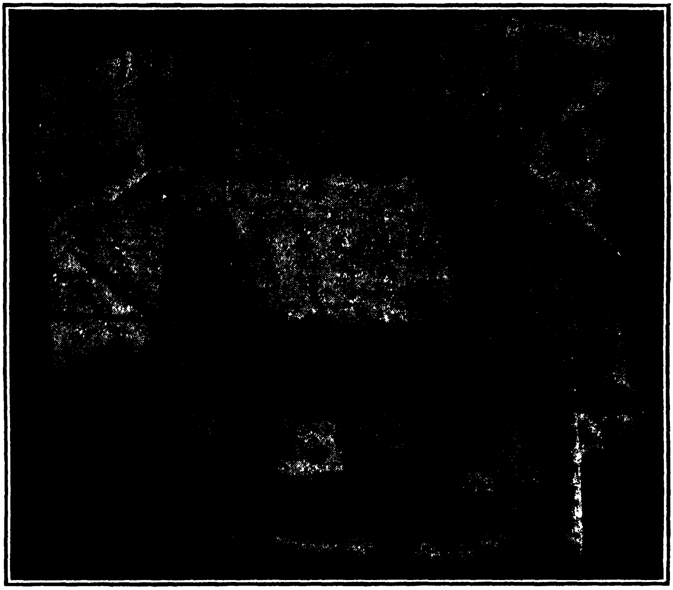

FIG. 39. Excessive mold development on pound print of butter.

Moldy butter. Development of molds occasionally occurs with both print and tub butter. Growth is largely confined to portions at or very near the surface, owing to the oxygen requirements of the organisms, although rarely an air space may permit some growth in the butter mass. Mold on butter is the cause of considerable loss because the moldy portion must be removed, thus involving a decrease in weight and additional labor, and also because of the influence of the mold growth on the flavor of the normal-appearing portion. The defect is most common during the warm months when the temperature is especially favorable for growth. In general, moldy butter is an indication of unsatisfactory methods of manufacturing and/or packing. However, various factors, such as high holding temperatures, may be the immediate cause of the condition; unsatisfactory car refrigeration repeatedly has resulted in moldy butter reaching the markets in large quantities. There appears to be a great variation in the prevalence of mold in butter from year to year as well as from season to season.

Types of molding. The moldy areas that develop on butter vary widely in appearance, owing to differences in the species involved and also in the extent of growth. In some instances two or even more molds may be growing in combination, and this associative action has a pronounced influence on the character of the growth.

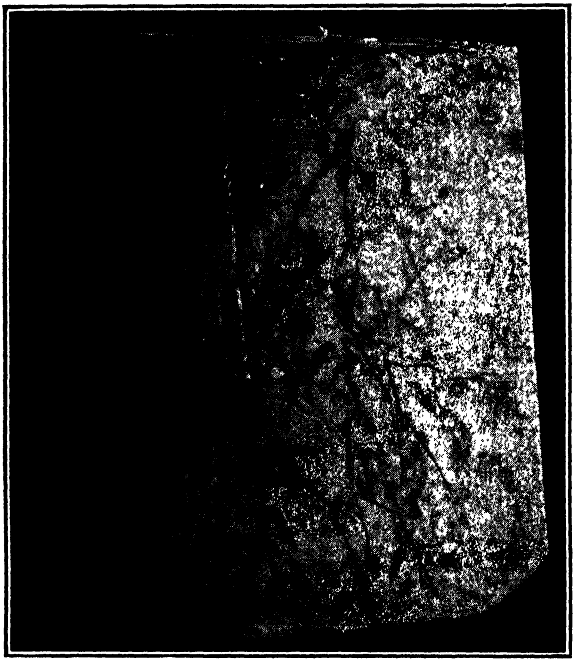

FIG. 40. Mold development on tub butter. Courtesy H. Macy.

Thom and Shaw [138] recognized three main types of mold growth:

1. Smudged or *Alternaria* type. In this type of molding there are dark, smoky, or rarely greenish-colored areas suggesting soot or dirty finger marks. Sometimes hyphae were observed 4 or 5 mm below the surface. Spores were rarely found but colonies transferred to culture media grew freely and fruited normally. Dark brown or black hyphae were the most common and proved to be species of *Alternaria;* from greenish-colored areas species of *Cladosporium* developed.

2. Green type. Green molds sometimes develop on the surface of butter and follow seams or cracks down into the butter mass. The mold also may be present on the liners, circles, or tubs. Cultures obtained from this type of molding were species of *Penicillium.*

3. *Oospora* type. Various shades of orange and yellow discoloration showing little or no surface growth also develop on butter. Cultures and microscopic examinations showed that this condition was due to *O. lactis.* The organism grew to a depth of several millimeters within the mass of butter as a complex mycelium with hyphae that varied in diameter according to the size of the spaces between the masses of fat. Some spores were formed and at times there was surface fruiting.

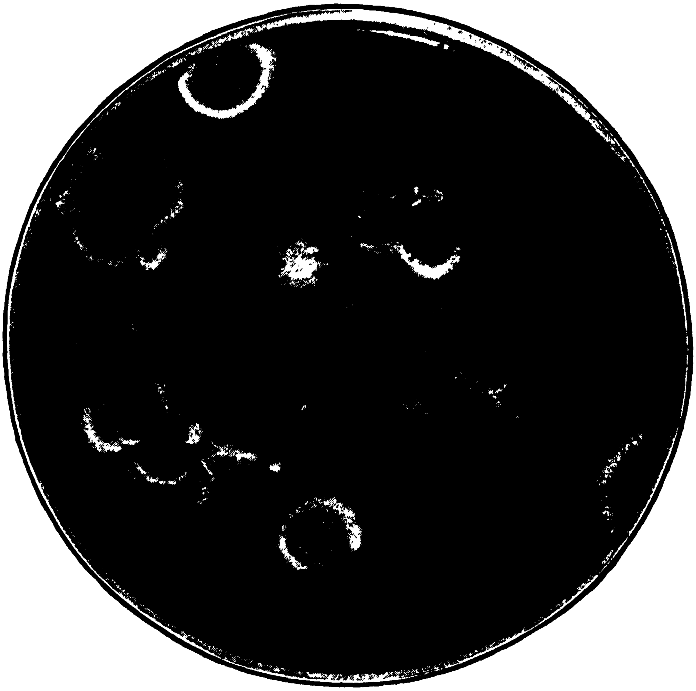

FIG. 41. Mold development following the covering of contaminated parchment with agar.

Sources of molds in butter. Molds in butter can come from various sources as indicated by the general sources of organisms in this product.

Macy and Combs [139] carried out field studies on the sources of molds in Minnesota creameries and found that the raw cream was heavily contaminated, even when most of it was sweet and fresh. About 90 per cent of the dry parchment and cloth circles was contaminated. In nearly 75 per cent of the creameries the piping and pumps were adding molds to the cream; in more than 65 per cent the churns were infected. Water was a source of molds in 44 per cent of the plants, culture in 40 per cent, and salt in 33 per cent.

Hood and White[140] found that the interior of moldy butter contained large numbers of yeasts and molds which indicates that the butter must have been heavily contaminated. In a comparison of butter made from raw and from pasteurized cream, the investigators found no appreciable difference in the contents of yeasts and molds, owing undoubtedly to extensive recontamination of the pasteurized cream.

Preventing contamination with molds. Production of cream free from molds is impossible under practical conditions but proper pasteurization controls the contamination of butter from this source. The studies of Thom and Ayers[141] show the general resistance of mold spores to heat. When heated for 30 min in milk, a number of species were killed at 54.4 C (130 F), while a few were destroyed at a lower temperature. Several species survived at 60 C (140 F), but 62.8 C (145 F) was found to destroy mold spores completely enough to render them a negligible factor in the future changes in milk. With a heating period of 30 sec, 62.8 C killed very few species, 68.3 C (155 F) killed most species, whereas 73.9 to 79.4 C (165 to 175 F) killed practically all, although some species occasionally survived. Dry heat was much less effective; with a 30-min period, 82.2 C (180 F) killed very few species and some even survived 121.1 C (250 F). From these results it is evident that the molds in cream are readily destroyed by proper pasteurization. Ineffectiveness of the pasteurization in certain creameries from the standpoint of mold destruction is shown by the results of Macy and Combs,[139] who found the molds had not been destroyed in 40 per cent of the samples of supposedly pasteurized cream from Minnesota creameries.

Preventing contamination of the pasteurized cream and the butter with molds involves attention to the various sources from which organisms can gain entrance to these products. The equipment, particularly the churns, often has added many molds to cream and butter. In certain cases it is advisable to treat wrappers, liners, circles, and tubs in such a way that any molds present are destroyed. Contamination from the air should be limited as much as possible by exposing the cream and butter to the air for the minimum period.

Riddet and Neill[142] noted that molds originating in butter boxes may infect the surface layers of butter packed therein and cause damage. A double layer of 28/30 lb parchment did not prevent mold mycelia from penetrating the surface of butter from the internal surface of butter boxes but aluminum-foil parchment did prevent such penetration. Immersion of butter box timber for 10 min in a 0.1 per cent aqueous solution of sodium salicylanilide appeared to be an effective method of preventing mold growth in butter boxes.

Growth of molds in butter. Contamination of butter with mold is not necessarily followed by growth and many lots of butter showing excessive mold counts have a normal appearance for extended periods. More or less definite requirements must be met before growth occurs, and these are not always provided in butter.

Molds grow on a variety of materials, many of which are comparatively resistant to action of organisms, so that the nutrients supplied by butter undoubtedly are satisfactory. The fat is less suitable for growth than the curd, and some of the butter constituents that are present in very small amounts may be especially important in this connection. The salt present in butter has an inhibiting effect on mold development, as is shown by the fact that mold is much more troublesome with unsalted butter than with salted. Early studies of Gripenberg[143] recognized the importance of salt in controlling mold growth, and all subsequent investigations have emphasized it. Thom and Shaw[138] concluded that 2.5 to 3 per cent salt in butter, which is equivalent to 12 to 15 per cent brine, is sufficient to eliminate mold or reduce it to a negligible amount. However, investigations have shown that many lots of butter containing these amounts reach the markets in a moldy condition. Hastings'[144] explanation was that amounts of salt which effectively prevent germination of mold spores do not stop growth of vegetative cells; accordingly, if pieces of mold filaments are present in or on butter, as a result of contamination from some source, they may continue their development in the presence of considerable salt, although salt undoubtedly decreases the rate of growth. Boekhout and Ott de Vries[145] also noted the restraining action of salt on mold cultures from moldy butter.

Temperature has a very direct influence on development of molds. In general, growth is progressively reduced as the temperature is decreased from room temperature, until at 0 C (32 F) it is very slow if it occurs at all. Under practical conditions rapid development of molds takes place principally when the butter is held at temperatures that are comparatively high for storage.

High humidity favors growth of molds, as is evident from the frequent development of these organisms in damp places, such as cellars. Inoculations made from moldy butter to normal butter in a petri dish often fail to grow under room conditions, but show good development in a moist chamber. Moisture content of the air appears to be more important than that of the material on which the molds are growing, and with a favorable humidity molds may develop on nutrients that are very low in water. Thom and Shaw[138] found that molds would not grow on butter exposed to humidities of 70 per cent, or lower, whereas a species of *Penicillium* grew on a mixture of butter fat and skim milk powder held at a favorable humidity. Air spaces in butter packages have an opportunity to develop a high humidity because the air in them is more or less confined and can take up water from the butter and moist packing materials, especially when the butter does not retain the moisture properly. Removal of butter to a lower temperature raises the relative humidity of the air spaces.

Molds require free oxygen for their development and, accordingly, mold growth in butter is confined to portions where air is available. Occasionally, growth penetrates several millimeters from the surface in contact with an oxygen supply. Cracks left by careless packing may be a factor in mold development because the air supply which they provide favors both initial mold growth and extension of that already begun. Butter, especially if it is leaky, tends to collect water next to the tub and when this drains away air spaces of considerable size may result. Paraffining of tubs has its advantage, in part, in preventing loss of water, either through absorption by the wood or actual drainage, and thus controls formation of air spaces.

Mold inhibitors. Various chemicals have been suggested for control of mold growth on butter and on parchment in contact with it. Propionates have received special consideration in this connection because of their effect in controlling mold growth on bread and also because of their natural presence in certain dairy products.

Ingle[146] reported tests on unsalted butter stored at 15.6 C (60 F) and 100 per cent humidity which indicated that wrappers containing either 6 per cent calcium propionate or 12 per cent sodium propionate greatly inhibit growth of mold on the surface of butter. Wetting propionate-impregnated wrappers before wrapping the butter gave better results than using the impregnated wrappers dry. There seemed to be little difference in action between parchments treated with sodium and calcium propionates. Olson and Macy[147] found that parchment paper treated in 5 per cent calcium propionate solution acidified to *p*H 5.5

with lactic acid was fully as effective in inhibiting mold growth on unsalted butter as parchments treated in 10 per cent unacidified solution; the butter wrapped with the former papers showed marked superiority in keeping qualities to butter wrapped with the latter papers. There was no advantage in using a solution consisting of a mixture of either propionate with sodium chloride for the treatment of wrappers. Solutions of either propionate were superior to a saturated sodium chloride solution for inhibition of surface mold growth. Calcium-propionate-impregnated parchments were effective with dry wrapping of butter but immersing such paper in water before the wrapping leached the propionate from the paper. Heating propionate solutions did not impair their effectiveness. The *Penicillium* species used was markedly more resistant to the inhibiting effect of propionate than species of other genera. The major role in inhibiting growth of the molds studied was ascribed to undissociated propionic acid molecules.[148]

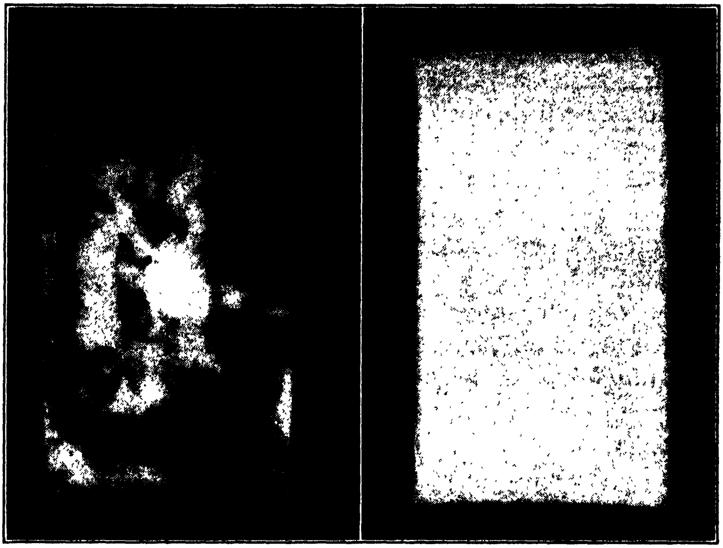

FIG. 42. Effect of mold inhibitor on butter artificially contaminated with mold. *Left:* control. *Right:* wrapped in parchment treated with 10 per cent solution of calcium propionate. Courtesy H. Macy and J. C. Olson.

Propionates also have an effect on certain bacteria. Mann and Frazier[149] showed that sodium and calcium propionates readily inhibited development of *E. coli* and *Alc. viscosus* and exercised considerable restraint on *S. lactis*, but *S. thermophilus* and *L. bulgaricus* were more resistant; there was comparatively little effect on *O. lactis*. Wolford and Andersen[150] investigated the action of propionates in controlling microbial growth in fruits and vegetables and found the inhibitory action of sodium propionate on micro-organisms varied with *p*H of the substrate, being greater at low *p*H values. Concentrations which destroy some organisms at *p*H 3.5 to 4.5 may not retard their growth at *p*H 6 to 7. Under practical conditions sodium propionate significantly delayed microbial spoilage in fresh figs and berries; it delayed such spoilage in fresh lima beans and peas even with the more nearly neutral reaction.

There should be little objection to use of propionates in connection with food products because of their production, through fermentation, in various dairy products, particularly swiss-type cheese. When Harshbarger[151] fed calcium or sodium propionate to growing white rats in quantities up to 3 per cent of the diet, there was no decrease in the gain in weight.

The fatty acids offer some interesting possibilities in connection with inhibition of molds on various products.[152,153] Butyric acid appears to have good fungistatic properties and the same is true of certain other acids. The objectionable flavors of some of the acids higher than propionic is of importance in the case of food products; also, their low solubilities in water may interfere with their efficient use.

Color changes due to pink yeasts. In some few instances pink yeasts have been responsible for a color change in butter. This appears either as a pink tinge over a considerable area or as small sharply defined spots. Investigations have shown that at least some of the pink yeasts develop better in unsalted butter than in salted.

Bacterial surface discoloration. In Great Britain[154] Hiscox reported surface discoloration of mildly salted butter that was cut into pats and held in cold storage awaiting distribution. Two strains of bacteria producing bluish-black pigment were isolated from the discolored areas.

Samples of Canadian print butter showing black to reddish-brown discoloration in irregular patches on the surface were investigated by White.[155] The discoloration penetrated to a depth of about one-eighth inch. Microscopic preparations made from the discolored portion revealed many small Gram-negative rods. An organism capable of causing the defect was isolated by plating the butter on nutrient agar with 1.5 per cent sodium chloride added, or by streaking on slopes of the same medium, incubation being at 4 or 15 C (39.2 or 59 F). It produced blackish, insoluble pigment on culture media at temperatures of 4 and 15 C with low concentrations of salt; optimum conditions for pigmentation appeared to be 4 C with 1.5 to 2.5 per cent salt. The defect was produced in butter by inoculating the cream used for churning and also by inoculating the butter directly, either with wash water infected with the organism or during the working process, provided 0.5 per cent salt was added to give about 3 per cent salt in the serum. Experimental butter, when unsalted or with over 1 per cent salt to give a salt concentration of over 6 per cent in the serum, did not develop the discoloration. Low concentrations of salt appeared to be essential for proper growth and characteristic pigmentation by the organism but with over 5 per cent salt in broth culture media, or in butter serum, pigmentation was inhibited and growth retarded. The organism was named *Pseudomonas nigrifaciens*.

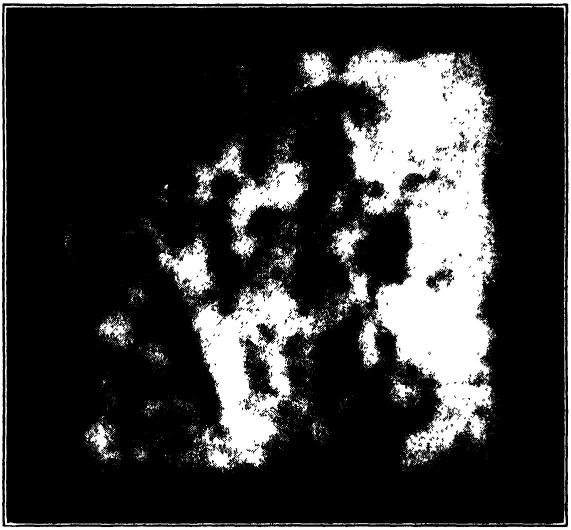

FIG. 43. Surface discoloration of butter due to *Pseudomonas nigrifaciens*. Courtesy A. H. White.

Butter showing discoloration through growth of *Ps. nigrifaciens* has been encountered in various sections of the United States. Commonly, *Ps. nigrifaciens* can be isolated by plating on a salt medium. On one occasion the defect was encountered in putrid butter and both *Ps. nigrifaciens* and *Ps. putrefaciens* were isolated.

Color changes not due to organisms. Color changes have been noted in butter due to causes other than action of organisms.

Pink color due to sulfur dioxide. Hammer and Olson[156] studied print butter, from two widely separated sources, that showed areas of distinct pink color at the surface. Microbiological investigations indicated the butter contained neither unusual numbers nor types of organisms. When portions of the butter that were normal in appearance were exposed to sulfur dioxide a pink color quickly developed. The butter from each source had been held in a mechanical refrigerator using sulfur dioxide as a refrigerant and, in the one instance in which an examination was made, a leak permitted the gas to escape into the food compartment. Butter from each source developed a pink color when exposed to various reagents, including a number of acids. The butter was made with certified color. Experimental butter colored with either yellow AB or yellow OB developed a pink color when exposed to various reagents, but with the yellow AB the pink color developed more quickly and was more pronounced than with the yellow OB.

Surface darkening. The surface of butter sometimes shows a darkening of the normal yellow color that is especially noticeable when the surface color is compared with the color of the interior. The defect occurs most often in prints, particularly when there has been an opportunity for drying, and is considered to be due to loss of moisture from the surface layers. In New Zealand, Barnicoat[157] noted that cold-storage butter often assumes a deep tint that is described as "primrose" color. It is not due to freezing since fresh butter at room temperature can develop the defect. Barnicoat concluded that the color is due to a surface desiccation that alters the structure and appearance of the butter, just as the opposite effect may be observed during its manufacture, when the incorporation of water and the working cause the butter to assume a lighter color. For control, he suggested use of a wrapper that is impermeable to moisture and effective sealing.

KEEPING-QUALITY TESTS ON BUTTER

The attempts that have been made to establish a correlation between the numbers of various types of organisms in butter and its keeping qualities have not been successful. Many lots of butter containing large numbers of organisms of different species deteriorate only slowly; others with much smaller numbers deteriorate rapidly. This is due to variation in the effects of various species on butter and also to the influence of physical condition, salt distribution, etc., on the growth of organisms in it. The importance of satisfactory keeping qualities in butter has led to the suggestion that small portions of butter be held under conditions very favorable for spoilage, and examined frequently for appearance of defects. In general, the defects this procedure is expected to detect are those due to organisms rather than those due to chemical action.

Bouska and Brown[158] reported that when small samples of butter are stored at 15.6 to 21.1 C (60 to 70 F) the lots that will keep poorly develop a bad flavor within 3 days.

Sutton[117] used a bottle test to detect certain butter defects. About 30 gm of butter was placed in a cotton-stoppered, sterile Erlenmeyer flask (200 ml capacity) that was then partially submerged in a water bath at 40 to 50 C (104 to 122 F) and agitated until all the butter was melted. A sterile cork was substituted for the cotton stopper, the flasks incubated at 15 to 20 C (59 to 68 F), and examined after 1 and 7 days. Normally, no odor developed when the butter originally contained less than 100,000 bacteria per gram, as shown by plates poured with beef extract agar and incubated 2 days at 37 C (98.6 F) plus 2 days at 15 to 20 C, but an odor developed when the butter contained more than 500,000 bacteria per gram. In specific instances an odor developed in butter containing less than 100,000 bacteria per gram. The method was particularly valuable for detecting a defect described as decomposed. The melting of the butter in the method suggested by Sutton introduces a variation in the physical state that makes the conditions quite different from those in commercial butter.

Jacobsen[159] noted that when unsalted butter and serum from it were held at 21 C or room temperature more rapid and extensive flavor deterioration and more rapid increase in numbers of bacteria occurred in the serum than in the corresponding butter. A study of flavor deterioration in unsalted butter serum at 21 C did not aid materially in the prediction of the keeping qualities of the corresponding butter at 21 C; flavor defects appeared sooner in the serum than in the corresponding butter but frequently were quite different from those produced in the butter.

Various butter organizations have found keeping-quality tests useful in improving the marketing of their products. There is considerable variation in the procedures employed. Small portions of butter from churns or tubs can be placed in sterile glass containers; wrapped prints also are used, and these can be taken from the print table in order to have the butter more representative of the condition in which it is marketed. In some instances the butter is held at 21.1 C (70 F); in others a somewhat lower temperature, such as 15.6 to 18.3 C (60 to 65 F), is employed. The butter is examined after various holding periods but usually the first examination is made after 2 or 3 days because of the great advantage in detecting, as soon as possible, the lots of butter that will not keep. The final examination is made after 7 to 14 days. When subjected to keeping-quality tests many lots of butter become tallowy because of the great effect of temperature on development of this defect, and ordinarily such a change is ignored in the tests.

Keeping-quality tests have been considered too sensitive by certain users and not sensitive enough by others. The former group believes that certain lots of butter deteriorate rapidly at the temperatures used for the tests and keep satisfactorily under commercial conditions; the latter group believes that some lots of butter keep satisfactorily in the tests and still deteriorate under commercial conditions. Temperature has a definite effect on the growth of organisms in butter, and a close correlation between deterioration at various temperatures cannot be expected. Extensive commercial experience indicates that, with suitable pasteurization and minimum contamination of cream and butter, it is possible to manufacture butter that keeps satisfactorily, as far as biological defects are concerned, even under very adverse conditions. Keeping-quality tests are helpful in detecting faulty methods of production, and their use is advisable until more adequate methods are devised. In employing the tests each sample of butter must be examined critically; one defective churning among many that are satisfactory is very easily missed. General experience indicates that before a serious outbreak of spoilage occurs in the product of a plant there frequently is a period during which an occasional churning shows poor keeping qualities; if these are detected and corrective measures instituted, an outbreak may be avoided. Normally, with butter that is to be printed it is highly advisable to test the printed rather than the unprinted product because of the effect printing often has in so changing the condition of the butter that bacterial spoilage is favored.

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Chapter 17

Bacteriology of Cheeses

The possibility of preparing cheese from milk apparently was discovered by accident when milk was held in a container made of the stomach of a calf, where it was exposed to the action of rennin. More or less definite types of cheeses originated through variations in temperatures used in manufacture, water and salt contents, and ripening conditions, since all these factors have a pronounced effect on the species of organisms developing in the product and, accordingly, on the chemical changes induced. The fat content also has a conspicuous influence on the final product. Although methods of making cheeses were developed long before the importance of organisms in causing changes in dairy products was recognized, study of the action of various species in cheese ripening has resulted in improvements in the manufacturing procedures and has made it possible to produce successfully various cheeses in localities that lack some of the natural advantages under which the making began.

The changes involved in the ripening of certain cheeses constitute the most complex microbiological processes in the field of dairying. Action of large numbers of organisms of various species is required and it often is difficult to evaluate the exact role of the different species. Study of the ripening processes is greatly complicated by the presence in cheeses of organisms to which it is difficult to assign a definite significance but which, nevertheless, may be of importance. The numerous unsolved problems in connection with cheese ripening indicate the difficulties presented.

In addition to the natural cheeses, processed cheeses, cheese spreads, and cheese foods are used in large amounts.

Classification of cheeses. Many varieties of cheeses are recognized[1] but often the differences between two or even several varieties are insignificant and there are only relatively few basic types. With most varieties, rennin in the form of commercial rennet extract is used to coagulate the milk, and a portion of the water and materials in solution in it are drained away as whey. Cheeses can be divided into hard, semihard, and soft groups, depending largely on the moisture content of the finished product. Hard cheeses are primarily bacteria ripened, semi-hard cheeses are primarily bacteria or mold ripened, and soft cheeses are primarily bacteria or mold ripened or unripened; however, enzymes from the milk used or added with the rennet sometimes play a part in the ripening. The classification in the table following includes the important commercial varieties.

Important Commercial Varieties of Cheeses

General Group of Cheeses	*Variety of Cheese*	*Primary Ripening Organisms*
Hard	cheddar	bacteria, not including eye formers
	swiss	bacteria, including eye formers
Semi-hard	brick	bacteria
	blue	mold
	roquefort	
	gorgonzola	
Soft	limburger	bacteria
	camembert	mold
	cottage	unripened
	cream	
	neufchatel	

With the typical hard cheeses ripening goes on quite uniformly throughout the mass, except as influenced by such factors as drying at the surface, and if desired they can be made in large sizes. The properly made and ripened products are not susceptible to rapid action of objectionable organisms and, as a result, have good keeping qualities. With the ripened soft cheeses micro-organisms prominently connected with the ripening develop at the surface and the products of growth diffuse through the cheese mass and effect the characteristic changes; accordingly, the necessary diffusion limits the sizes of the cheeses. With these cheeses the opportunity for continued growth of micro-organisms, even after the desired ripening has occurred, results in development of various defects and the keeping qualities are limited. In the unripened soft cheeses flavor development occurs during manufacture. Although there may be an increase in the pleasing flavor for a period after the cheese is made, extensive growth of organisms commonly results in loss of the desired flavor and in formation of abnormal flavors; yeasts and molds find conditions favorable for their development and often cause serious defects. The semi-hard cheeses require definite ripening through the action of organisms and, in general, they rank between the hard and the soft groups in keeping qualities.

General ripening agents. The general changes that occur in cheeses during ripening involve action of micro-organisms and of enzymes normal to milk or added with the coagulating agent. More than one species of organism ordinarily is concerned with the ripening of a variety and the changes brought about by the various species may be quite different. Products resulting from the action of one ripening agent sometimes make possible a later change, and in this way a regular sequence of changes may occur. Ripening of a cheese always is a complex process because of the many transformations involved and the details are difficult to evaluate.

Some of the early work on cheese ripening led to erroneous conclusions as to the causative agents. Bits of old cheese were inoculated into sterile milk and yielded coagulation and a cheesy odor that led to the assumption that spore-bearing rods developing in the milk were an important factor in the ripening. These organisms apparently belonged to the aerobic spore formers that so commonly produce a cheesy odor in milk; they controlled the fermentation in the inoculated milk because the bacteria that are important in the ripening are not spore formers and do not survive the conditions existing in old cheese in such a way that they can grow rapidly when introduced into sterile milk. Later investigations showed the presence of large numbers of acid-forming bacteria during ripening of various cheeses and indicated the importance of such types. In general, the organisms that grow well in milk also grow well in cheese, and normally the first step in the making and ripening of various cheeses is development of acid through action of bacteria, usually one or more of the lactic acid streptococci. To ensure an abundance of acid-producing streptococci in cheese, a lactic acid culture commonly is added to the milk. Coagulation of milk with rennet or acid concentrates these organisms, as well as others, in the curd, and the whey commonly contains smaller numbers per milliliter than the original milk. In laboratory trials Hastings *et al.*[2] found that three lots of milk contained 128, 11, and 2.05 million bacteria per milliliter, whereas the corresponding whey contained 34, 2.9, and 0.73 million. Under practical conditions three trials yielded counts of 5.6, 6.4, and 5.43 million per milliliter on the milk and 3.0, 1.29, and 2.4 million on the corresponding whey. In the laboratory trials about 77 per cent of the organisms were held in the curd and under practical conditions about 73 per cent.

Because organisms are concentrated in the curd the production of acid there is relatively rapid. Hastings *et al.*[2] presented data illustrating this point. Deep beakers were filled with milk, the milk curdled with rennet, the curd cut and allowed to settle, and then the acidity of the whey at the top and bottom determined after various periods, the beakers being held in a thermostat at 35 C (95 F) to eliminate convection currents. Acidities at the bottom of a beaker regularly were higher than at the top which indicates that acid production was rapid in the curd. This activity of the lactic acid organisms in the curd undoubtedly persists during the making and early ripening of various cheeses.

Distribution of organisms in cheeses. When milk is coagulated, with either rennet or acid, the organisms in the curd are held firmly, so that the growth that occurs later results in formation of colonies comparable to the colonies in a mass of agar. Accordingly, the organisms in a cheese are more or less unevenly distributed and, whereas a species that was very numerous in the milk is present in each tiny portion of the cheese, another type may not be present in a relatively large portion, even after extensive multiplication. Growth of an important organism may be definitely limited to a certain portion of the cheese by some such factor as air supply; this is best illustrated by the cheeses in which development of molds normally occurs. There is diffusion of materials to and from the colonies of organisms so that ripening is not confined to the immediate neighborhood of the masses of growth.

Determination of numbers of bacteria in cheese. The number of bacteria per gram of cheese ordinarily is determined by the plate method. The portion for examination is weighed on a sterile paper and ground in a sterile mortar with some material which helps to disintegrate it, after which the mass is transferred to a dilution blank. The blank is thoroughly shaken and used as with milk. Approximately 1 gm is a convenient sample but the exact quantity is immaterial, as long as the weight is known so that the final result can be calculated to a 1-gm basis. Ordinarily, the sample can be rather completely removed from the paper. If it cannot, the paper is ground with the cheese and then it is protected by having a larger paper on the balance pan.

A 3-in. mortar is a satisfactory size for grinding cheese. The mortar and pestle can be wrapped together in paper and heated in a dry sterilizer. Fine sea sand has been extensively used as a grinding agent and can be sterilized in cotton-stoppered test tubes. Sucrose has been employed occasionally and has the advantage of going into solution in the dilution blank; it can be sterilized by flooding with ether for several days, after which most of the reagent is poured off and that remaining allowed to evaporate. More recently, sodium citrate solution has been used. A 2 per cent aqueous solution can be sterilized and the desired quantity employed directly for disintegrating the cheese in the mortar. By adding the grinding material to the mortar in small amounts, the cheese can be broken up more easily than when it is added in one lot. The cheese must be very thoroughly divided and this requires extensive grinding.

If the dilution blank contains 1 per cent sodium citrate and a quantity of glass beads, disintegration of the cheese ordinarily is more complete on thorough shaking than when water is used. Appearance of the material in the dilution blank shows how completely the cheese has been disintegrated.

When possible, the sample of cheese for analysis is taken from a plug drawn with a sterile trier, after carefully scraping away, with a sterile knife or spatula, the surface portion of the cheese from the area to be bored. It is preferable to make up the sample from various places in the plug so that different parts of the cheese are represented, and in some instances cheese is taken from several plugs. The pieces of cheese used must be cut from the plug with a sterile knife and handled with sterile forceps to prevent contamination. Soft cheeses may be difficult to handle with a trier and then a sterile spoon or spatula is advisable; the surface should be avoided, because of the abundance of organisms there, unless it is of special interest when it is best examined separately. Blue-veined cheeses, in which there normally is an uneven distribution of the characteristic organism, are especially difficult to sample for microbiological examination.

Various media have been employed in plating cheese. Standard agar commonly is satisfactory for general counts but special media may be needed in examining cheese for certain species, including yeasts and molds. In studying the presence of organisms that are not definitely aerobic, shake cultures or plates with a limited air supply are necessary. With most cheeses, better colony development is obtained on plates at room temperature, 21 C (69.8 F), or 30 C (86 F) for 3 to 5 days than at 37 C (98.6 F) for a shorter period.

Because of the great difficulty in completely breaking up the colonies existing in a mass of cheese, values obtained in determinations of the numbers of bacteria regularly are too low. However, results obtained under controlled conditions on different lots of cheese or on the same lot at different periods show, in a general way, changes in both the numbers of organisms and the important types. If reliable data are to be obtained, disintegration of the samples must be thorough and uniform so that large clumps of bacteria will not be left undisturbed.

Variations in the moisture content of cheese make it advisable, in certain investigations, to run moisture determinations on material similar to that used for bacterial counts so that the results can be calculated to a moisture-free basis.

For rough determinations of the numbers of certain organisms, such as *L. casei,* that develop poorly on plates but well in milk, a method consisting of adding dilutions of ground cheese to tubes of sterile milk has been employed. The tubes are held for 1 to 2 weeks at a temperature favorable for growth and then examined in the gross and also microscopically in order to obtain information on the morphologic types in the various dilutions. Approximations of the numbers of organisms of the different types can then be made.

Valuable data on the organisms in cheese sometimes are obtained by making direct microscopic examinations. A piece of cheese with a smooth surface can be pressed firmly against a clean slide and then removed so as to leave organisms distributed on the slide in much the way they were on the surface of the cheese. The "impression" then is fixed, freed of fat with some such solvent as xylene, stained, and examined. For the purpose of showing that important morphologic types are not being missed by the plate method, a microscopic examination is as valuable with cheese as with other materials. Actual microscopic counts of the organisms in cheese can be obtained by making smears of the cheese dispersed in dilution blanks containing sodium citrate and beads, removing the fat with a solvent, staining, and counting.

Certain investigators have found it possible to cut sections of cheese for microscopic examination in the same way that sections of tissue are cut. Hucker[3] used such a procedure with cheddar cheese and noted that the numbers of organisms determined by this direct method were much larger than the plate counts.

CHEDDAR CHEESE

Cheddar cheese is a hard variety, originally made in England, in which normal ripening apparently involves action of various bacteria, without formation of eyes. The necessary bacteria multiply in the cheese and each general type brings about a change that is important from the standpoint of the ripening process. Other species of microorganisms commonly are present; certain of them are unimportant, whereas others may bring about undesirable conditions, such as off flavors, gas formation, etc.

Pasteurization of milk. Bacterial defects in cheddar cheese and, more recently, epidemics of disease, particularly typhoid fever, that have been traced to it emphasize the need for pasteurization of the milk used. The process was fist extensively employed with cheese milk in New Zealand and the cheese made proved satisfactory on the important markets of the world. Sawers,[4] in 1925, pointed out that pasteurization of milk made into cheese in New Zealand resulted in a product of a higher quality, which maintained its quality much better under storage than cheese made from raw milk, and which also stood transit better. The exposure suggested for pasteurization was 71.1 to 73.9 C (160 to 165 F) flash; if a lower temperature was used, not all the objectionable organisms were destroyed, whereas if a higher one was employed the curd lost its cohesive properties.

In the United States some of the early work on pasteurization of milk for cheddar cheese was carried out by Sammis and Bruhn;[5] the method involved heating to 71.1 to 73.9 C by the flash method and, after cooling, addition of hydrochloric acid to restore the coagulability of the milk. Price[6] found that pasteurization at 62.8 C (145 F) for 30 min produced better cheese than the other methods of pasteurization tried; the procedure involved addition of sufficient culture to produce 0.165 per cent acid in the whey at the time of dipping and sufficient rennet to give a curd firm enough to cut in 15 to 20 min. Pasteurization was most effective when the raw milk was of inferior quality but the quality of pasteurized milk cheese varied with the quality of the raw milk. Price and Prickett[7] investigated the effect of pasteurization of milk by various methods on the quality of cheese, using (1) raw milk, (2) milk flash pasteurized at 71.1 to 73.9 C and cooled immediately on a surface cooler, (3) milk flash pasteurized at 62.8 to 65.6 C (145 to 150 F) and conducted to a vat where it was not cooled until the entire lot had been heated, and (4) milk pasteurized at 62.8 C for 30 min. The bacterial efficiencies obtained, as averages of 10 runs, with the various methods of pasteurization were 98.31 per cent for the flash method, 98.65 for the flash-holding method, and 98.96 for the holding method. Although pasteurization by any of the methods improved the quality of the cheese, the flash method was not so effective as the other two. The yield of cheese from pasteurized milk was 2.5 to 4 per cent greater than the yield from identical milk not pasteurized, owing to retention of more moisture.

Wilson *et al.*[8] reported observations indicating that the quality of milk is one of the most important factors, if not the most important, in determining the quality of cheddar cheese; they noted that the detrimental effect of inferior quality in the milk can be partly overcome by pasteurization, which destroys the lactic acid and gas-producing bacteria and permits the cheesemaker to control the fermentation in the vat, but that it does not remove entirely the effects that objectionable bacteria produce in milk prior to pasteurization. They found that the beneficial effects of pasteurization may be entirely nullified by so accelerating fermentation of the lactose that it is exhausted and the point of maximum acidity reached by the time the cheese is removed from the press. Results showed that the amount of culture should be limited to an extent that will ensure a slow but gradual fermentation, so that at milling (not less than 4.5 hr after setting) *p*H of the curd will not be below 5.30 and not much above 5.50, preferably between 5.40 and 5.50. When the cheese comes from the press about 24 hr after setting, it should still contain an appreciable amount of lactose for continuance of the fermentation. The slow, gradual fermentation with pasteurized milk of good quality avoids the acid and bitter flavors that are in much of the cheddar cheese commonly made.

Walter and Lochry[9] noted that extensive use of pasteurization in the making of cheddar cheese had demonstrated that a uniformly high percentage of No. 1 cheese can be made from pasteurized milk with a suitably controlled amount of active culture and a definite time schedule, the latter simplifying the cheesemaking operation.

Establishing an acid-producing flora. At the time the rennet is added to the milk, active lactic acid streptococci should be present. This species ensures an adequate acid production during the manufacture and early stages of ripening that is necessary if the normal changes are to occur. A lactic acid culture commonly is added to the milk and the organisms permitted to grow for a short period before coagulating with rennet.

Changes in numbers of bacteria during early ripening. After the curd goes to the press the lactic acid streptococci continue to grow actively. Because of the difficulty in breaking up clumps of bacteria in cheese, the plate counts that have been reported undoubtedly are too low but they indicate the general trend in the changes in numbers of bacteria early in the ripening. Harrison and Connell,[10] employing the plate method with beef peptone gelatin or peptone whey gelatin, usually found the highest bacterial count on normal cheese when it was 1 day old, although occasionally the maximum occurred in 2 to 5 days; the highest number of bacteria noted was 635,000,000 per gram. Lactic acid organisms regularly predominated. Harding and Prucha[11] also obtained data with the plate method indicating that the largest number of organisms occurs early in the ripening process. *S. lactis* was the only species always found and it made up more than 99 per cent of the total organisms. Hastings *et al.*[2] noted that the maximum number of bacteria, as measured by lactose agar plates, occurred early in the ripening; in 8 of the 11 cheese examined, the largest number of bacteria was found within 48 hr of making. Some counts over 1,000,000,000 per gram were obtained and the maximum was 2,518,000,000. In studies on the influence of lactic acid streptococci on the chemical changes in cheese ripening, Kelly [12] found the numbers of bacteria increased rapidly in certain lots to as many as 100,000,000,000 per gram after 10 to 13 days and then decreased somewhat less rapidly; other lots showed much lower maximum counts.

Because of the influence of various factors, there undoubtedly is considerable variation in the maximum number of organisms in different lots of cheese and in the time this maximum occurs. Lactose greatly favors growth of *S. lactis* and the amount of it retained is influenced by the moisture content of the cheese. It is probable that during early ripening the maximum number of organisms is reached at about the time the lactose disappears. The temperature of holding would be expected to influence the rate of growth of organisms. Dolby *et al.*[13] found that the quantity of lactose incorporated in cheese is of importance in determining the acidity. When the lactose content was increased by adding it to either the milk or the curd, there was a more acid type of cheese at maturity although some lactose was still present in the cheese after 4 mo.

Following the early increase in numbers of bacteria, as determined by the plate method, there is a decrease that is rapid at first but much slower later. The decrease principally involves *S. lactis* and is due to development of conditions that are unfavorable for this species. Occasionally, *S. lactis* is found in very old cheese.

Importance of acid development. Under normal conditions *S. lactis* is the first species to bring about an important change during the making and ripening of cheddar cheese. This change consists primarily of transformation of the lactose to lactic acid. Production of acid begins early in the making process and normally continues as long as sugar is available. Because of the relatively high buffer capacity of cheese in relation to the sugar content, the *p*H change ordinarily does not prevent the growth of *S. lactis* with lactose still present. Lactic acid is important in the manufacture and ripening of cheddar cheese because of the following five effects: [2]

1. *Favors curdling of the milk with rennet.* Activity of various enzymes is strikingly influenced by reaction of the medium and, accordingly, more rapid coagulation of milk with rennet is obtained when there is some developed acid present.

2. *Favors expulsion of the whey.* Proper expulsion of the whey is necessary to reduce the moisture content of the cheese to a satisfactory point. If the moisture content remains too high, the cheese commonly has an off flavor and fails to develop the desired character. Rapid production of acid aids materially in expelling whey by causing contraction of the curd. Sammis *et al.*[14] found that, with the percentages of acid in normal cheese curd, expulsion of whey was proportional to the amount of acid present.

3. *Favors fusion of the curd particles.* Fusion of the curd particles is necessary if the cheese is to have the proper body. The particles must lose their identity and form a compact mass, rather than show a tendency to retain their forms. Proper fusion is favored by acid and, without it, the cheese often is crumbly.

4. *Exerts a protective action against putrefaction.* Although putrefactive bacteria of various types often are present in cheese, normally they are prevented from growing largely because of the acid present. Babcock *et al.*[15] noted that, when curd was washed free of acid, and of sugar from which acid could be formed, it rather quickly decomposed as a result of action of putrefactive organisms.

5. *Favors action of the pepsin in the rennet extract.* Rennet extract contains the proteolytic enzyme pepsin in addition to the coagulating enzyme rennin. Pepsin plays a part in the breakdown of the casein derivatives and its action is favored by the presence of acid produced by the lactic acid organisms. This action can be readily observed by adding about 2 ml of sterile milk to each of two tubes of melted agar, inoculating one with a very small amount of *S. lactis* culture, and then emptying each tube into a sterile petri dish. After incubating so that the organisms in the one dish have had an opportunity to grow, a narrow strip of filter paper moistened with rennet extract is placed on each agar surface; both dishes then are held at 37 C (98.6 F). In a short period the dish in which *S. lactis* has produced acid will show a clear area around the paper, because the pepsin in the acid medium has changed the casein to soluble compounds, whereas the dish in which there has been no acid formation will show little if any clearing.

If a distinction is made between *S. lactis* and *S. cremoris* it apparently makes little difference which organism is employed in cheesemaking. Kelly[16] concluded that acid production appears to be the important function of the cultures used in cheddar cheese and that satisfactory cheese can be made by inoculating the milk with either *S. lactis* or *S. cremoris.* In commercial cheese made without culture, and also in cheese made using a culture of either *S. lactis* or *S. cremoris,* about 50 per cent of the flora was made up of *S. lactis.*

Lactobacilli. The lactobacilli constitute another group of organisms of importance in cheddar cheese. They may reach numbers of 1 to 2 billion, or more, per gram. The lactobacilli grow so poorly on certain media, particularly when growth must start from a single cell or small clump instead of from a mass of cells, that they probably have been missed or underestimated in various studies on cheese.

The comparative numbers in which *S. lactis* and the lactobacilli are found in a cheese at different ages is illustrated by the following data obtained by Hastings *et al.:*[2]

COMPARATIVE NUMBERS OF *S. lactis* AND LACTOBACILLI IN A CHEESE AT DIFFERENT AGES

Age of Cheese, Days	*S. lactis* Percentage	Lactobacilli Percentage
14	99.99	0.01
22	99.90	0.10
37	90.90	9.10
45	99.90	0.10
77	99.00	1.00
100	90.90	9.10
155	50.00	50.00
165	1.00	99.00
187	1.00	99.00

The results show that, from the standpoint of numbers, *S. lactis* is important early in the ripening period and the lactobacilli are dominant later.

Conditions favoring growth of lactobacilli. Growth of lactobacilli in cheese occurs, for the most part, at a period when the lactose has been used by *S. lactis;* presumably, in cheese, as in milk and cream, *S. lactis* is capable of more rapid development than the lactobacilli, so that growth of lactobacilli largely is limited to a period when conditions have become unsatisfactory for *S. lactis.* Addition of peptone to milk favors acid production by various cultures of lactobacilli, and peptone probably also favors the growth of these organisms in cheese. Since the changes occurring in the ripening of cheese result in formation of various protein decomposition products, including peptone, development of lactobacilli in cheese at a period when sugar no longer is available may be accelerated by the accumulation of protein decomposition products.

The sequence of changes that apparently leads to active development of lactobacilli relatively late in the ripening period is (1) production of lactic acid by *S. lactis,* (2) activity of pepsin as a result of the favorable reaction resulting from this acid, (3) formation of protein decomposition products by the pepsin, and (4) stimulation of the lactobacilli by these products.

Another factor of importance may be associative action of organisms. Hansen[17] noted that stimulation of *L. casei* by *S. lactis* or *S. cremoris* may play a role in the ripening of cheeses in which the two latter species develop, die, disintegrate, and are followed by *L. casei.*

Changes produced by lactobacilli. Presumably, the most important change effected in cheese by the lactobacilli is in connection with the breakdown of protein. Growth of the organisms in milk results in an increase in the soluble nitrogen that often is considerable on a percentage basis, especially with extended incubation, and the same general change probably occurs in cheese. The large numbers of lactobacilli in cheese indicate that these organisms induce rather extensive changes.

Allen and Knowles[18] concluded tentatively that at least three factors are essential in inducing proper ripening in cheese made from milk of low bacterial count: (1) a vigorous cheese culture, (2) lactobacilli of a type that will extend the protein decomposition initiated by enzymes and streptococci, and (3) an adequate source of nitrogen to allow growth of the culture organisms and lactobacilli. Lane and Hammer[19] found that with six of eight strains of *L. casei* added individually to pasteurized milk made into cheese there was a more rapid and extensive decomposition of the protein than occurred in cheese made from pasteurized milk without added lactobacilli; two strains had little effect on the decomposition. Flavor scores of the cheese made from pasteurized milk inoculated with *L. casei* commonly were more uniform and higher than those of either raw- or pasteurized-milk cheese. Three of the strains of *L. casei* gave a typical cheddar cheese flavor while three others gave a pleasing, butter-like flavor, suggestive of biacetyl, that was not typical of cheddar cheese. Phillips[20] noted that, in cheese having a *p*H value as low as 5.0 at 4 days, there was an improvement in flavor when a lactic acid culture was supplemented with lactobacilli.

Sherwood[21] found that, when inoculated into milk for cheddar cheese, different strains of lactobacilli imparted specifically characteristic flavors to the cheese; these included both desirable and undesirable flavors, many of which commonly are encountered in commercial cheese, such as pleasant aromatic, biacetyl-like, metallic, bitter, unclean, and fermented. Inoculation of cheese milk with natural mixtures of lactobacilli from mature cheese almost always led to development of unclean, fermented flavors. Later, Sherwood[22] reported that a significant feature of the ripening of New Zealand cheddar cheese is the early displacement of the lactic acid streptococci by lactobacilli. When clean milk (plate count after pasteurization $<2{,}000$ per milliliter) is employed, lactobacilli usually make their appearance approximately a fortnight after manufacture, but in commercial cheese the organisms dominate the flora earlier, frequently attaining very high numbers when the cheese is a few days old. Sherwood[23] also noted that improvement of cheese by inoculation of good-quality milk with selected strains of lactobacilli gave the best results when relatively small inoculations were employed. Large inoculations gave a sharpness to the flavor during the early stages of ripening and this sharpness often persisted when the cheese was mature. The most convenient method of propagating lactobacilli under factory conditions consisted of the incorporation of suitable strains of lactobacilli into the lactic acid cultures (enriched with a vegetable extract). The strains grew better in association with some lactic acid cultures than with others.

According to McDonald,[24] development of mature flavor in cheddar cheese can be hastened by adding cultures of *L. casei* during manufacture. The increased rate of flavor development was most marked when whole milk cultures were added to the cheese in the later stages of manufacture but the flavors that developed as a result of this technic were not uniform and off flavors may occur. The best-controlled improvement in rate of development of mature flavor took place when whey was used as the basic medium (various additions being made) for growth of cultures of lactobacilli, but no means could be devised to make the whey-base medium a practical procedure.

Use of lactobacilli. The difficulties in classifying the lactobacilli isolated from cheese and comparable materials complicate the study of their value in cheese ripening. Moreover, two strains that belong to the same species might have very different effects on the flavor of cheese; this situation obtains in the cases of *S. citrovorus* and *S. paracitrovorus* in lactic acid cultures and probably with other organisms producing flavor in dairy products. The large numbers of lactobacilli in many lots of fine raw-milk cheese suggest that these organisms are of importance in the ripening, and the trials in which cultures of lactobacilli have been used in cheese milk indicate that beneficial results should be obtained if selected strains are used under suitable conditions.

Additional organisms. In addition to *S. lactis* and lactobacilli, various organisms are found more or less frequently in cheese. Certain of them have a desirable influence on the ripening, others are inert, and still others are definitely objectionable because they induce defects.

Streptococci other than *S. lactis* appear to be of importance in developing flavor in cheese. Organisms of this general group are necessary in the production of the desired flavor in lactic acid cultures. Evans[25] found two species of streptococci, which were designated *Streptococcus x* and *Streptococcus kefir,* common to various ripening cheeses; these organisms and another unclassified *Streptococcus,* either singly or in combination, improved the flavor and hastened the softening of cheddar cheese made from pasteurized milk. Hucker and Marquardt[26] noted that addition of *S. paracitrovorus* to milk, either alone or in conjunction with cheese cultures, appeared to have a desirable effect on the flavor of cheese; a flavor not unlike that of raw-milk cheese developed in cheese made from pasteurized milk into which this organism had been inoculated. The investigators reported that *S. citrovorus* appeared to have no effect on the flavor of cheese made from milk to which it had been added. Hansen *et al.*[27] found that, when a milk culture of *S. paracitrovorus* was added to raw milk in amounts less than 4 per cent, the cheese had a bitter flavor and open body and texture. With pasteurized milk a more desirable cheese was obtained but when the amounts of culture were from 4 to 10 per cent a bitter flavor resulted. When a milk culture of *S. citrovorus* was used in amounts from 1 to 10 per cent the cheese had a bitter flavor and an open body and texture.

In the studies of Hart *et al.*[28] streptococci and micrococci isolated from cheddar cheese produced large quantities of volatile acid, especially acetic, when grown in milk; the milk used was practically free from fat so the acids must have come from such sources as citric acid, lactose, and protein. At times these cocci are present in cheese in large numbers, so they were considered to be a factor in the volatile acid production that occurs during ripening.

It is probable that different organisms may be involved in the production of flavor in different lots of cheese, all of which have a fine flavor. Although *S. lactis* and lactobacilli regularly are present, constituents that are important from the standpoint of flavor may be produced by one species, or group of species, in one cheese and by some other species, or group of species, in another. In this way variations occur in the organisms present in satisfactory cheese and study of the ripening process is complicated.

Protein decomposition. From the chemical standpoint one of the important changes in the ripening of cheese is the breakdown of the casein derivatives. The tough, green curd must undergo extensive protein degradation before it becomes a product that is recognized as ripened cheese. This decomposition is effected by enzymes and bacteria; the enzymes are protease and pepsin, the latter being present in the rennet extract, and various organisms are involved.

Protease increases the soluble nitrogen in milk held with a reagent that prevents bacterial growth, and this suggests that protease is important in the protein decomposition during the ripening of cheese. Many of the investigations of this enzyme have been stimulated by the cheese-ripening problem, but the exact influence it has in cheese is difficult to determine because of the activity of other proteolytic agents. With protease, protein decomposition goes to the amino acid stage and considerable nitrogen in this form is one of the characteristics of various hard cheeses. The acid reaction in these cheeses is not so satisfactory for the action of protease as a neutral or slightly alkaline one.

During the early studies on cheese ripening there was a difference of opinion as to whether rennet extract has an effect in addition to coagulating the milk. Trials at the New York (Geneva) Agricultural Experiment Station [29] indicated that with an increase in the amount of rennet used there was an increase in the soluble nitrogenous products formed. Results of Babcock *et al.*[30] show this same effect and also that the increase in the products formed is confined to the higher decomposition bodies peculiar to pepsin, thus suggesting that the digestive action of rennet extract is attributable to the pepsin incorporated with the rennet. In cheese made from milk to which purified pepsin had been added, either with or without use of rennet to curdle the milk, the protein decomposition was increased.

An acid reaction is important in connection with peptic digestion and, in cheese, this reaction is provided through activity of *S. lactis*. Acid salts, such as phosphates, favor peptic digestion in a manner comparable to free acids.[30]

Van Slyke *et al.*[31] made cheese from milk to which 4 to 5 per cent chloroform had been added and then held the cheese, containing 12 to 15 per cent chloroform, in an atmosphere of chloroform. The soluble nitrogen formed was less than in normal cheese and the proportion of albumoses and peptones was high in comparison with the amides, whereas in normal cheese the amides were high. The results show the effect of enzymes on the ripening process and also indicate that bacterial action is necessary for the normal changes. When 0.2 per cent lactic acid was added during the making of the cheese containing chloroform, the amount of soluble nitrogen was markedly increased, which suggests the importance of pepsin in the protein decomposition.

Riddet *et al.*[32] found that 5 oz of rennet, compared to 3 oz, per 1,000 lb of milk gave a more rapid breakdown in the earlier stages of cheese ripening and an increase in the proportion of soluble nitrogen, but there was a tendency to accentuate any off flavor and to develop a sticky body in the mature cheese. The trials indicated that a 30 per cent increase over the normal quantity of rennet would be an advantage when the cheese is to be sold for consumption soon after manufacture.

Additional investigations [33,34] indicate that a more rapid breakdown of cheese can be obtained with an increased amount of rennet. However, the flavor developed may be somewhat different from that occurring with the normal amount of rennet and longer ripening. The general results indicate that the problem of rapid ripening of cheese is not to be solved by increasing the amount of rennet used.

Among the organisms involved in protein decomposition in cheese, the lactobacilli are especially important. *S. lactis* also has the ability to increase the soluble nitrogen in milk and, presumably, does the same thing in cheese. With six commercial cheese Kelly [12] determined the protein fractions soluble in water, trichloroacetic acid, and phosphotungstic acid, as well as the amino nitrogen. A steady breakdown of the proteins was demonstrated by an increase in all the fractions. The amount of non-protein nitrogen (portion soluble in trichloroacetic acid) was never more than 24 per cent of the total nitrogen in the ripened cheese. With the exception of the protein fraction soluble in water, little change was noted for 10 to 13 days. The increase in protein digestion after about 10 days seemed to be related to the destruction of bacteria; it may be that proteolytic enzymes are released that aid in the protein hydrolysis.

Davis *et al.*[35] reported that in milk the enzymes of commercial rennet, in conjunction with the lactic acid bacteria in cheddar cheese, can bring about protein breakdown similar in extent to that found in ripened cheese, as far as the non-protein nitrogen is concerned. The amino nitrogen produced is much less than in cheese which may be due to the higher *p*H in the cheese as compared to milk cultures, since acid adversely affects the peptidases present.

The general character of the protein decomposition in the ripening of cheddar cheese is shown by the early data of Van Slyke and Hart;[36] even with extended ripening, less than half the nitrogen was in water-soluble forms and amides made up a considerable portion of the water-soluble nitrogen. This type of protein decomposition would be favored by action of protease and certain bacteria rather than by action of pepsin.

The presence in cheese of specific amino acids has been shown by Tuckey *et al.*,[37] who found that, by the extraction of aged cheese in hydrated *n*-butyl alcohol, amino acids were extracted and could be identified with x-ray analysis. Leucine and iso-leucine were obtained from cheese 24 hr after being made. Tyrosine and probably tryptophane were identified in the extract obtained from ripened cheese. Dorn and Dahlberg[38] concluded that the white particles so frequently seen in ripened cheese are principally tyrosine, which previously had been found in roquefort cheese.[39] The white particles also have been identified as calcium lactate so they may be of various types.[40,41]

Acids, alcohols, and esters. During the making and early ripening of cheese, lactic acid increases rapidly, principally through action of *S. lactis;* later, there apparently is some destruction of it. Suzuki *et al.*[42] found that the amount of lactic acid fluctuated during the ripening but that some remained, even after an extended period. Lactic acid that developed long after the lactose had disappeared may have been of protein origin. The lactic acid in cheese usually was the *i* form; this may have been due to activity of the organisms in the lactic acid culture, since such a culture commonly contains considerable *i* acid. Presumably, the disappearance of lactic acid in cheese is due to action of organisms.

Succinic acid was isolated from ripening cheese and identified by its silver salt.[42] It also was obtained from a solution of lactose and peptone, fermented in the presence of calcium carbonate and fat, by inoculation of a small bit of cheese.

There appears to be a rise and then a fall in the volatile acidity during the ripening of cheese. Suzuki *et al.*[42] found that acetic and propionic acids reached a maximum at 3 mo and then decreased, whereas butyric and caproic acids increased continuously during the period of study. Formic acid was found once, at 5.5 mo, in whole-milk cheese and not at all in skim-milk cheese. Valeric acid was never obtained. Lactates were probably the principal source of acetic and propionic acids, while the fats and proteins were the sources of butyric and caproic acids. Ground cheese was distilled with steam after moistening it with dilute sulfuric acid, and redistilled following neutralization with barium hydroxide. The final distillate was characterized by an odor resembling that of cheese and it contained alcohols and esters. From whole-milk cheese the esters were largely of ethyl alcohol and acetic acid; from skim-milk cheese they were largely of ethyl alcohol and caproic and butyric acids.

Action of salt. The salt added to the curd affects the body and texture and also the ripening of cheese. Riddet *et al.*[43] noted that with undersalting cheese had a pasty and weak body, open texture, and did not ripen normally; with oversalting the cheese had a harsh body, ripened slowly, and the rind often cracked badly. McDowall and Whelan[44] reported that in milk growth of streptococci was inhibited in progressively greater degrees by salt concentrations from 3 to 6 per cent, and that complete inhibition was approached by any concentration above 6 per cent. Five per cent gave definite but incomplete inhibition; this concentration corresponds to the limit of salt concentration in cheese of good body, which suggests that the effect of high salt concentration on the body of cheese may be due to retarding the growth of lactic acid streptococci.

Immediately after salting, the surfaces and centers of pieces of curd have very different salt contents. With a microprocedure Hoecker and Hammer[45] found that at hooping the salt contents were relatively high at the surfaces of pieces of curd and comparatively low at the centers. During the early part of the pressing, rapid decreases in salt contents occurred at the surfaces and rapid increases at the centers; 24 hr after removal of the cheese from the press, differences in salt contents between the surfaces and centers of pieces of curd usually were small. The *p*H values at milling were essentially the same at the surface and centers of pieces of curd. For a time after hooping the *p*H values were lower at the centers than at the surfaces but the differences rapidly became less and 24 hr after removal of the cheese from the press they were small. The general results suggest that the effect of salt on the growth of organisms is relatively uniform throughout a cheese.

Importance of *p*H. The *p*H of cheese is of great importance from the standpoint of the quality of the product. In general, the *p*H increases during extended ripening because of the changes involved. Brown and Price[46] found a slight decrease in *p*H of cheese for some days after making and then a slow increase that became very definite with long ripening. They also noted that the quality of cheese is closely related to the acid development during the making; when the quality of the cheese is inferior, the *p*H is low and the titrable acidity high at each critical point. Other investigators [47] have noted the effect of low *p*H in decreasing cheese quality. Wilson *et al.*[8] emphasized the importance of *p*H control in the manufacture of cheese.

DEFECTS OF CHEDDAR CHEESE

A process requiring extended growth of desirable organisms from a flora as varied as that ordinarily present in raw milk would be expected to show irregularities and result in defects in certain lots of cheese. Some of these are due to activity of undesirable species and others to failure of the desirable types to develop normally. Often the defects are so slight they are of little importance; in other cases they are very objectionable and seriously lower the market value of the cheese. Introduction of pasteurization into the cheese industry has tended to control certain of the microbiological defects of cheese but inadequate pasteurization exposures, recontamination, etc., still permit irregularities.

Quality of milk. If raw milk made into cheese contains a variety of organisms in large numbers, species that produce undesirable changes may develop under the conditions provided. The necessity for growth of desirable organisms implies the possibility of development of objectionable species, and the manufacture of fine cheese requires milk that is satisfactory, bacteriologically. Some of the manufacturing and ripening methods tend to minimize the action of undesirable organisms; for example, the salt added may restrain development of certain species.

The total number of organisms present is no indication of the quality of milk for cheesemaking. Large numbers of certain lactic acid bacteria may be desirable, whereas comparatively small numbers of a species capable of bringing about objectionable changes may be very serious. Investigations by Hucker [48] showed no striking relationship between the number of bacteria in the milk, as determined either by direct counts or by using the acidity as an index, and the score of the cheese, although it was noted that milk containing 12 to 42 million bacteria per milliliter produced cheese of more constant quality than did milk containing smaller numbers.

Pasteurization of milk for cheese destroys many of the objectionable organisms in the original milk but may not overcome the effect they may have had on the milk.

Lack of proper acid development. Comparatively slow acid development sometimes occurs during manufacture of cheese. Action of bacteriophage appears to be the primary cause but there are other possibilities. With slow acid development large amounts of moisture and materials in solution are retained and these afford an unusual opportunity for growth of objectionable organisms. An excessively acid flavor more or less regularly occurs in cheese having a high moisture content because of the abundance of lactose available, but other off flavors also appear. An abnormal acid development influences the texture of the cheese.

Gassy cheese. Development of gas holes is a common defect of cheese when raw milk is used and may occur with pasteurized milk as a result of inefficient pasteurization or recontamination. Normally, some gas is produced in a cheese but this develops so slowly that it has an opportunity to diffuse out as fast as it is formed and gas holes result only when excessive amounts are produced. Van Slyke and Hart[49] found that during ripening of a normal cheese carbon dioxide was given off continuously, although in decreasing amounts after about 20 weeks. The total amount produced was 15.099 gm, equivalent to 0.5 per cent of the fresh cheese. In a cheese made from milk containing chloroform and kept under antiseptic conditions, the total amount of carbon dioxide produced was 0.25 gm, practically none of which was formed after 3 weeks. The investigators considered that with the normal cheese the carbon dioxide given off early in the ripening came from decomposition of lactose by lactic acid organisms, whereas a small amount probably was attributable to gas present in the milk and to respiration of the organisms in the cheese. The carbon dioxide produced later apparently came from certain reactions that took place,

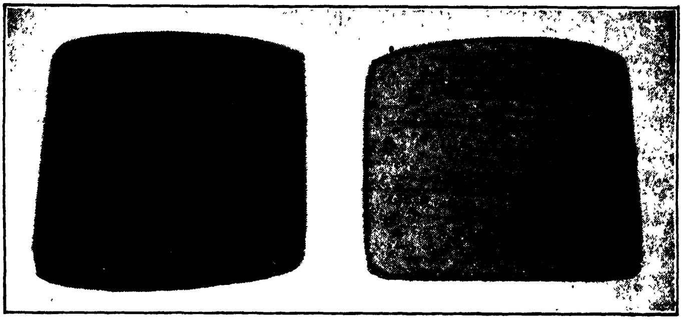

FIG. 44. Cheddar cheese showing gas formation.

among which was identified the change of tyrosine and arginine to derived products.

Dorn and Dahlberg [50] found that, in tins, cheddar cheese from pasteurized high-quality milk produced less gas than cheese from raw milk and also was more consistent from batch to batch. Gas evolution was more rapid at 10 C (50 F) than at 4.4 C (40 F); it was most rapid for approximately the first 4 weeks but never ceased even after 48 weeks. The gas was almost exclusively carbon dioxide, but one lot of raw-milk cheese gave a trace of hydrogen. There was no determinable amount of oxygen, methane, or ammonia. At 15.6 C (60 F), cheese removed nearly all the oxygen from a can in 1 week but at 4.4 C about 7 weeks were required. Evolution of carbon dioxide appeared to bear an inverse relationship to the loss of oxygen. Loss in weight of cheese due to carbon dioxide evolution varied from 0.13 to 0.20 per cent for raw-milk cheese and from 0.04 to 0.09 for pasteurized-milk cheese; all the milk was of high quality. The raw-milk cheese developed a little more flavor than the pasteurized-milk cheese; with both types the body and texture were very good.

Russell,[51] in 1895, isolated a number of organisms producing gas in milk and found, through inoculation experiments, that all of them had a marked effect on the texture of cheese, making it more or less spongy, and that the flavor invariably was poorer than that of the controls. In one trial development of gas was so pronounced the cheese huffed into a football shape and rolled off the shelf. The organisms apparently belonged to the *Escherichia-Aerobacter* group.

A gassy condition in cheese, which was accompanied by a definite off odor, was investigated by Moore and Ward [52] and found to be due to an *Escherichia-Aerobacter* organism. In the attempts to locate the source of the organism, milk drawn directly into sterilized jars under controlled conditions was found to contain it; this occurred both with the fore milk and with that drawn later in the milking. Udder infections with organisms of this type appear to be unusual.

Marshall [53] investigated cheese curd showing a marked inflation in the vat and isolated an organism that caused extensive gas development in cheese when added to the milk used in the manufacture; it apparently belonged to the *Escherichia-Aerobacter* group.

In an investigation of gas-producing bacteria and their effect on milk and its products, Harrison [54] found that the milk from 2 of 25 cows contained the organisms, even with every possible precaution against contamination from air and the coat of the cow, and concluded that gas formers occasionally come from the interior of the udder; other sources were found to be coat of the cow, milk cans, watering trough, flies, and manure. When the organisms isolated were inoculated into milk, the cheese made from it was typically gassy, the flavor was objectionable, and there was a mottled appearance that was thought to be due to action of the gas. Trials showed that the gas formers varied in the way they were restrained by cultures.

Sherwood [55] found that "open" cheddar cheese evolved carbon dioxide much more rapidly than "close" cheese. Lactobacilli and betacocci capable of producing carbon dioxide relatively rapidly could be isolated from open cheese and addition of such organisms to milk for cheese resulted in development of slit openness in the cheese. The most common types causing slit openness appeared to be gas-producing lactobacilli.

Yale and Marquardt [47] made coliform counts on 35 lots of cheese, taking samples of milk at setting, curd at milling, and cheese at different ages; after 3 days the cheeses were cured at 10 C (50 F). Maximum coliform counts on six lots of cheese made from poor-quality raw milk (coliform counts from 20,000 to 2,100,000 per milliliter) ranged from 9,900,000 to 410,000,000 per gram and the organisms survived 6 to 12 mo. With better milk containing fewer coliform organisms, they survived 3 to 6 mo. The pasteurized milk used contained <1 coliform organism per milliliter but coliform counts on cheese curd ranged from 900 to 57,000 per gram because of recontamination and growth, even with special precautions. Gas holes developed in the cheese when the coliform count exceeded 10,000,000 per gram, provided no attempt had been made to control the gas. Acid development to *p*H 5.0, or less, greatly inhibited growth of coliform organisms but resulted in cheese with acid defects.

Although gas formation in cheese commonly is due to *Escherichia-Aerobacter* organisms, lactose-fermenting yeasts have been involved in some instances and spore-forming anaerobes in others. Rapid acid production during the making of cheese tends to restrain certain gas formers but may not entirely prevent their activity.

Off flavors. Various off flavors are encountered in cheese and a number of them are caused by micro-organisms. Most of the off flavors that develop in milk and cream also are found in cheese. Composition of the cheese may be a factor in the development of certain of them; for example, some off flavors due to organisms are more common when the moisture is high and the acidity low.

Bitterness. Various micro-organisms can produce bitterness in cheese; bitterness varies from only a slight defect to one that is very conspicuous. Apparently, the condition also can be caused by irregularities in the making process.

In an outbreak of bitterness in cheese described by Harrison,[56] a bitter condition occasionally was noted in the milk as it was dumped into the weigh can but was more evident when the milk was warmed for setting and increased as the curd was heated. When the curd was very bitter, the top of the whey showed small gas bubbles, especially on heating. The bitter flavor was permanent in the cheese. There was a variation from day to day in its extent, and it sometimes was accompanied by an aromatic or fruity flavor. A yeast that produced a bitter taste in milk was isolated from samples of bitter curd. Other yeasts also were present, some of which gave pleasant, ethereal odors in beer wort. Samples of milk were taken from individual farms and, of 99 samples from as many patrons, 97 contained yeasts, several species often being present; the predominant species, the one producing bitterness in milk, was named *Torula amara.* The organism repeatedly was isolated from can washings and whey, and also was found on the leaves of certain maples. Ten min at 62 C (143.6 F) killed it but it was not destroyed in a can when the can was scalded with water at about 93.3 C (200 F). Gas was produced in milk. In 5 or 6 hr at 37 C (98.6 F) inoculated milk had a bitter flavor, and after 14 hr the flavor and aroma were disagreeable and strong; in 10 days at 37 C the milk was thick and slightly acid. Butter made from cream into which the organism had been inoculated showed a pronounced, bitter, disagreeable flavor.

A bitter flavor in cheese has been ascribed to *S. paracitrovorus* [27] and to acidoproteolytic cocci; *S. liquefaciens* also appears to be of importance. Presumably, bitter products of various types can be produced in cheese by a variety of organisms. The type of organism responsible is not the only factor involved; the number also is significant and if their numbers are small certain species can be present in cheese without causing bitterness.

Phillips [20] found with various lactic acid cultures that, when the *p*H of the cheese was 4.90 at 4 days, bitter flavor subsequently developed; it appeared in a majority of the cheese with a *p*H of 4.95, in a part of the cheese with a *p*H of 5.00, but not in cheese of higher *p*H. Within the limits of salt contents used, no relationship was found between bitterness and salt content. Price [57] noted that bitter flavor in cheese seemed to be associated with development of a slightly excessive amount of acid; he suggested that limiting acid development during the curd-making operations should afford an excellent means of reducing the incidence of bitterness.

Unclean flavors. Apparently, a variety of organisms can cause unclean flavors in cheese, just as in milk, with *Escherichia-Aerobacter* types of special importance. Whitehead[58] reported that representative *Escherichia-Aerobacter* organisms produced unclean or cowy flavors in cheddar cheese when added to flash-pasteurized milk immediately before manufacture of the cheese. They did not produce gas holes in the cheese, even when present in large numbers, provided the milk was inoculated with an active culture. Whitehead considered it probable that the metabolism of the organisms was modified by the lactic acid streptococci so that, although they grew, little or no gas was produced. The texture of the cheese was not influenced.

Rancidity. A rancid flavor in cheese can be caused by organisms but many of the common lipolytic bacteria are sensitive to acid and their growth is inhibited in cheese. The condition also can be caused by milk lipase, especially when its activity is influenced by certain conditions.

In a study of rancid flavors in cheese Hood and White[59] found that introduction of undesirable bacteria into the milk produced the defects in experimental cheese. They concluded that bacteria which produce rancid flavors can enter cheese from poor-quality milk, improperly sterilized equipment, poor cultures, milk cans used to return unpasteurized whey to the farms, contaminated water, and insanitary conditions in the factory.

Hlynka *et al.*[60] produced rancid and other less defined flavors in cheddar cheese by addition of commercial lipase to the milk. With added lipase a higher flavor score was obtained in cheese when larger amounts of pepsin and/or rennet were used in the manufacture than when smaller amounts of proteolytic enzymes were added; this suggests that proteolytic enzymes may enhance cheese flavor by preventing rancidity due to lipase. In whey-fat mixtures prepared by homogenization, Gould[61] found that rennet extract in amounts from 3 to 20 oz per 1,000 lb, when used either to precipitate the casein or when added directly to rennet whey, did not exhibit marked inhibitory effects on lipase activity. Hlynka *et al.*[62] reported that rancid and unclean flavors in cheddar cheese were fundamentally related and were caused by activity of milk lipase. The lipase was believed to be activated by vigorous agitation of uncooled cheese milk. The longer the agitation of experimental lots of cheese milk, the lower the score of the resulting cheese; the lower the temperature at which the milk was agitated, the better the flavor score. The results suggest that minimum agitation together with cooling are desirable in producing milk for cheese.

Later,[63] it was noted that milk which was churned for 15 min at 30 C (86 F) showed fat dispersion roughly equivalent to that in milk containing 20 per cent homogenized milk.

Miscellaneous flavors. Growth of various organisms in cheese influences the flavor of the product. The flavor may be slight and indefinite and of relatively little importance or it may be conspicuous. In some instances the flavor produced is due to action of a combination of organisms.

A malty flavor due to *S. lactis* var. *maltigenes* is encountered in cheese and may be very pronounced. A putrefactive flavor results from protein decomposition in which the products have objectionable flavors. Certain organisms that produce objectionable flavors in milk are prevented from growing in cheese because of the inhibitory effect of the *p*H.

Harding *et al.*[64] investigated a class of flavor defects designated as "sweet" and concluded they were due to yeasts. Cheese of normal flavor contained very few if any yeasts, whereas cheese showing the sweet flavor contained large numbers, at least during the first stages of ripening. Addition of cultures of certain yeasts to the milk repeatedly caused off flavors in the cheese, some of which were included under the general term of sweet flavor.

Color changes in cheese. Various color irregularities have been noted in cheese, some of which are due to micro-organisms.

Rusty spot. Harding and Smith[65] studied development of red or rust-colored spots in cheese made in New York factories and found that the organism responsible in all cases was one which Connell[66] had isolated in Canada. The spots were very fine and quite evenly distributed throughout the cheese, although they were most noticeable on the surfaces of gas holes and other openings; from 4 to 8 days were usually required for their development. Texture and flavor did not appear to be influenced by development of the color. Although cheese made from the milk of individual patrons indicated that at times the milk coming to a plant contained the organism, the main source of the organism appeared to be factory contamination. Thorough steaming of all the utensils and equipment, together with good factory sanitation, brought the defect under control. Return of whey to the farms may have been a means of scattering the organism in the barn surroundings. Davis and Mattick[67] also isolated the organism causing rusty spot in cheddar cheese. They found it to be an anaerobe that formed pigment only under certain conditions and regarded it as one of the true lactic acid bacteria that produces pigment under favorable influences. These investigators[68] listed the conditions that appeared to govern growth and pigmentation by the organism. Later,[69] it was reported that the most important factor in growth of the organism in milk is the presence and growth of certain other bacteria. The organisms most active in metabiotic stimulation of pigment production were those inducing low oxidation-reduction potentials in milk. There was no correlation between *p*H or other properties of ripened cheese and the extent of rusty spot. Pederson and Breed[70] reported that rusty spot in cheddar cheese is caused by chromogenic varieties of at least two distinct types of lactobacilli, namely, *Lactobacillus plantarum* var. *rudensis* and *Lactobacillus brevis* var. *rudensis.* The authors pointed out that the two species belong to the two main groups of species in the genus *Lactobacillus;* the first forms only a trace of carbon dioxide in its production of lactic acid from glucose and other carbohydrates, whereas the second produces, in addition to lactic acid, carbon dioxide, alcohol, and volatile acids from dextrose, and mannitol from levulose.

Pink color due to yeast. Mason[71] reported results on the discoloration of cheese made on the wensleydale system. The cheese was normal in appearance when first cut but became pink soon afterward, the color appearing along fine cracks and veins in the cheese. The color penetrated only to a depth of about 0.5 mm and the tinted portion could be sliced off, leaving a normal surface that in turn became pink. A yeast was isolated which gave a pink color in various media. By adding the organism to milk, a pink color was produced in cheese made so that the curd was soft; in cheese made on the cheddar system no pink color resulted, probably because of the texture of the curd.

Special color defects. Hood and White[72] described an unusual color in Canadian cheese shipped to England. When cut or bored the defective cheese exhibited varied intensities of color ranging from normal to pink, reddish, brown, and muddy shades; white cheese showed dirty brown, muddy shades. Chemical tests indicated the presence of nitrates or nitrites in the cheese, and on bacteriological examination nitrate-reducing organisms capable of producing color changes in annatto nitrate milk were isolated. The typical color defect developed in experimental cheese made from milk inoculated with mixed cultures of organisms when potassium nitrate was added to the salt used for the curd. The defect appeared in 7 weeks, whereas in cheese made from milk inoculated with the organisms but not treated with potassium nitrate the color still was normal after 3 mo. The nitrate apparently was added to cheese in certain Canadian factories to prevent objectionable fermentations. Harrison[73] also isolated nitrate-reducing organisms from the discolored cheese. He suggested that such organisms may be present in potassium nitrate and the water used. *Ps. fluorescens* is a water organism capable of producing discoloration in milk with added annatto and potassium nitrate.

Hood and White[74] examined cheese that showed light brown to yellowish brown spots on a freshly cut surface. The spots varied in size from a pin head to 0.5 in. in diameter; many of them contained a hard black core, and the centers of others were smudgy black. After bacteriological examinations showed no unusual flora, microscopic study revealed small pieces of wire in many of the discolored areas. They evidently came from steel wool used in cleaning the cheese vats.

Changes in the pigments of annatto may be involved in certain color changes in cheese. Barnicoat[75] reported that in development of bleached and pink discoloration in cheese an oxidation may be involved. Later,[76] he noted that pink discoloration in cheese can be produced by removal of some of the yellow component of annatto, since it was shown that (1) annatto acts as an indicator, being reddish in acid solution, (2) it tends to precipitate in acid solution, (3) the acid-precipitated pigment on the casein of cheese is more red than the same strength dye in alkaline solution, and (4) oxidation preferentially destroys the more yellowish, unprecipitated fraction, leaving the more red precipitated dye, which gives a pink appearance to cheese.

Molds. Cheese provides materials that are very satisfastory for the nutrition of molds and, since spores are so widely distributed, development of these organisms occurs when the requirements as to air, humidity, and temperature are met. Because of the air supply necessary, mold growth occurs mainly at the surface of cheese; it may be partly controlled by a coat of paraffin but cracks in the paraffin, which often are due to collection of moisture beneath, afford an opportunity for growth to start and it then may extend rather rapidly. Special types of waxes which are flexible and adhere well to the cheese are helpful in controlling mold growth and reducing loss of moisture from the cheese. In some instances mold growth follows openings down into the cheese, and the plugging of cheese commonly results in development of molds along the opening and the returned plug. Humidity and temperature of storage rooms for cheese commonly are suitable for mold growth so that limiting the air supply is the principal means of control.

Contamination of the surface of cheese with molds often occurs as a result of their growth in the curing room; this is especially serious when molds are growing on shelves used to hold the cheese, but also may occur through spores that are in the air.

Mold inhibitors. Use of mold inhibitors has been considered in connection with cheese, especially pieces cut for sale, since mold growth on them often is very troublesome. Miller [77] reported studies on mold inhibition on cheese cuts wrapped in moisture-proof coverings. Immersion of cuts in 8 per cent propionic acid solutions increased the mold-free life at 15.6 C (60 F) from the usual 3 to 5 days to 12 to 38 days, depending on the cheese and method of treatment. Solutions of 14 to 16 per cent calcium or sodium salts were required for similar effects. Extent of contamination, period of immersion, and period of draining all influenced the results. Concentrations of propionic acid up to 40 per cent did not entirely eliminate mold growth. Ingle [78] studied the effect of propionates on cuts of natural cheese wrapped in tinfoil, moisture-proof cellophane, or pliofilm, the cheese being dipped in propionate solutions or the wrappers being sponged with them. Samples dipped in 8 per cent propionic acid remained free of visible mold about twice as long as the controls; with 8 per cent calcium or 8 per cent sodium propionate the samples kept somewhat better than the controls but did not approach those treated with propionic acid. Sponging the wrappers had little effect in holding down surface mold.

Smith [79] found that ultra-violet light emitted by a 15-watt, low-pressure, mercury-vapor lamp did not prove effective in decreasing air-borne mold spores in a cheese curing room. Direct irradiation failed to prevent mold growth on the surface of the cheese.

CLARIFICATION OF MILK FOR CHEDDAR CHEESE

Removal of most of the dirt and some of the bacteria from market milk by clarification suggested use of the process with milk intended for cheddar cheese. Fisk and Price [80] found that, regardless of the quality of the milk, clarification improved the quality of cheese compared to the control from unclarified milk, and the cheese kept better in storage. Combs *et al.*[81] concluded that clarification of milk of poor quality under ordinary plant conditions gave no increase in the score of cheese, whereas when extreme care was used in the plant there was an increase of 1.59 points; with milk of high quality, clarification under ordinary plant conditions resulted in an increase of 2.53, whereas when extreme care was used in the plant there was an increase of 2.83.

Wilster [82] studied the influence of clarification on the quality of cheese made from 67 lots of milk. With cheese 1 mo old clarification resulted in an increase of 0.07 point in the average flavor score, whereas with cheese 3 mo old the increase was 0.41; in the former cheese clarification did not influence the average score on body and texture but in the latter cheese it caused an increase of 0.15. The influence of addition of slime obtained from 40 gal of milk to 20 gal of clarified milk intended for cheese was studied in six trials, using unclarified milk from the same lots as controls. The slime caused an average decrease of 1.08 points in the total score in 1-mo-old cheese and of 1.25 in 3-mo-old cheese. In five comparisons addition of an alkali-forming organism to clarified milk caused an average decrease of 1.20 points in the total score of 1-mo-old cheese and of 1.25 with the 3-mo-old cheese, compared to the score of cheese made from unclarified milk of the same original lots.

Hood and Hlynka [83] found that clarification of milk for cheese resulted in (1) removal of sediment, (2) improvement in flavor and texture of cheese, and (3) reduction in fat loss in whey. Vats of clarified milk developed acid at a faster rate and were ready to set earlier than vats of unclarified milk.

ATTEMPTS TO ACCELERATE RIPENING OF CHEDDAR CHEESE

Although some cheese are used very young, with little attention being given to the flavor, others are ripened for considerable periods. Various attempts have been made to develop more desirable flavors during ripening and also to reduce the ripening period and thus the cost of ripening.

Special bacterial cultures. The importance of bacteria in cheese ripening and the difficulty in establishing the particular role of different species have led to tests with organisms other than the lactic acid culture species and lactobacilli.

Hansen [84] noted that addition of 0.05 per cent of a milk culture of *S. liquefaciens* or 0.5 per cent of a milk culture of an unidentified *Micrococcus* to pasteurized milk improved the flavor of cheese made from it but did not materially influence the nitrogenous decomposition; a lactic acid culture and *L. casei* also were added to the milk. *Pseudomonas fluorescens, Alc. viscosus,* and *Ach. lipolyticum* had little effect on the flavor of cheese but *Ps. fragi* decreased the flavor score.

Harris and Hammer [85] found that 34 *Micrococcus* cultures, originally isolated from cheddar cheese, had the following effects on the flavor when inoculated into pasteurized milk used for cheese: 7 had undesirable effects, producing bitter and unnatural flavors; 14 had no effects; and 13 had desirable effects. Strains apparently belonging to the same species differed in their effects. When used in small numbers, certain strains of propionic acid bacteria improved the flavor of the cheese, whereas others did not; in large numbers all the strains improved the flavor and some produced a distinct sweet flavor somewhat resembling the flavor of swiss cheese. Babel and Hammer [86] previously had reported that about 85 per cent of 54 samples of cheddar cheese of varying qualities contained propionic acid bacteria in 0.1 gm; usually there were <5,000 per gram, but a few samples, all from one producing section, contained over 1 million. The numbers of the organisms were not related to the age or quality of the cheese.

Deane *et al.*[87] added various cultures to milk intended for cheese and noted an inconsistency in the results. The most promising results were obtained with an acidoproteolytic organism (isolated from old cheese) and a commercial culture. In two trials, the combination produced cheese in which the flavor was improved and the period required to reach the maximum flavor score was significantly shortened, compared to the controls. With the acidoproteolytic organism no significant off flavor, beyond that recognized as slight, was detected in the cheese 5 mo after preparation and 3 mo after reaching a quality definitely superior to cheese made with regular culture from the same lot of milk. In the one trial with it, *L. bulgaricus* had an undesirable influence on the cheese throughout the entire ripening period.

Higher curing temperatures. With cheese made from raw milk, curing temperatures often are kept relatively low to avoid rapid action of organisms that may cause objectionable conditions. Use of pasteurized milk largely overcomes this hazard and permits attempts to speed up the ripening by the use of higher temperatures.

Riddet *et al.*[32] reported that the effect of holding cheese at 15.6 C (60 F) compared to 7.2 to 10 C (45 to 50 F) was evident in the increase in soluble nitrogen values and in the more rapid maturing of the cheese. It appeared that holding an extra 2 weeks at 15.6 C during the early stages of ripening was more effective than holding an extra 6 weeks at 7.2 to 10 C subsequent to the normal 2 weeks in the factory curing rooms at 15.6 C. All the cheese held at the higher temperature had more open texture but good cheese closed up to some extent as it matured. Freeman and Dahle [84] noted that proteolysis in cheese ripening could be increased 40 to 100 per cent by raising the ripening temperature from 7.2 to 17.2 C (45 to 63 F); the maximum flavor score was reached more quickly at 17.2 C and was as high as at the lower temperature.

In studying the effect of ripening temperature on the quality of short-cure Texas cheese, Hanson *et al.*[33] found that 15.6 C gave the most desirable results and that up to 21.1 C (70 F) gave satisfactory results. Rate of proteolysis was increased by increasing the temperature; proteolysis could be checked by moving the cheese to a lower temperature. According to Deane *et al.*[87] a preincubation at 17.2 to 18.3 C (63 to 65 F) for 4 weeks followed by curing at 6.1 to 7.2 C (43 to 45 F) usually induced a quicker maximum flavor score than continuous curing at 6.1 to 7.2 C but the cheese did not always maintain so high a flavor score as the control. Sanders *et al.*[88] found cheese made from pasteurized milk of good quality was as fully ripened in 3 to 4 mo at 15.6 C as that cured for 6 mo at 10 C (50 F) and developed better flavor.

Use of high curing temperatures presents some problems. Erekson[89] noted that curing cheese below 4.4 C (40 F) affords the following advantages compared to curing at 15.6 C: (1) There is no loss from insects, (2) shrinkage is kept at a minimum, (3) special humidity control in commercial storage is seldom necessary, occasional circulation of air being sufficient, (4) maximum use of storage space is obtained, and (5) cheese can be held 1 to 2 yr without handling and with a minimum selection of original stock and minimum deterioration in grade. He listed the advantages of high-temperature curing as (1) cheese with a special sharp flavor can be produced, and (2) the flavor can be produced in 3 to 4 mo with unpasteurized-milk cheese. A high flavor also can be obtained with pasteurized-milk cheese but it is not comparable to that of raw-milk cheese.

Special proteolytic enzymes. Use of special proteolytic enzymes in cheese manufacture offers some interesting possibilities from the standpoint of accelerated ripening. Freeman and Dahle[34] reported that addition of pure rennin increased the rate of proteolysis in cheese ripening, increased slightly the rate at which flavor developed, and produced an aged product with a slightly higher flavor score. Added pepsin also increased the rate of proteolysis, particularly at the beginning of the ripening and at the lower ripening temperatures. It did not accelerate development of flavor but produced an aged product with appreciably higher score. Added trypsin increased markedly the rate of proteolysis during the early part of the ripening, after which its effect was greatly reduced. The enzyme also increased the rate at which flavor developed but reduced the maximum flavor score.

Babel *et al.*[90] obtained a cheese with a soft waxy body by using an enzyme preparation from chicken proventriculi to coagulate the milk and then following a modified edam process in the making. The active proteolysis was accompanied by development of a bitter flavor, which later tended to disappear.

It appears that various enzymes can accelerate the increase in soluble nitrogen in cheese but that the problem lies in finding an enzyme that gives the correct type of decomposition products. Commonly, the products are relatively complex and do not approximate the simple products normally formed in cheddar cheese.

Increasing fat hydrolysis. There have been various suggestions that fat hydrolysis plays a part in flavor development in cheddar cheese. The early studies of Suzuki *et al.*[42] indicated the possibility of fat hydrolysis accounting for the fatty acids obtained from cheese. Lane and Hammer[91] noted that in aged cheese considerable flavor suggestive of ripened cheese was contained in the fat. Acid numbers of fat from fresh (2-day) cheese were relatively high, compared to those of fat from sweet-cream butter, and increased with ripening; values for raw-milk cheese were higher when the cheese was fresh and increased more rapidly than those for pasteurized-milk cheese. Volatile acids of raw-milk cheese regularly were higher than those of pasteurized-milk cheese during the 200 days of ripening; values for the former usually decreased slightly after 90 days, whereas those for the latter continued to increase. Cheese[92] made from raw homogenized cream mixed with skim milk (raw or pasteurized) commonly developed a rancid flavor early in the ripening but the condition tended to disappear as ripening progressed and, eventually, the cheese was not rancid and was better in flavor than cheese made from pasteurized milk or pasteurized homogenized cream mixed with pasteurized skim milk. Cheese made from pasteurized milk had low fat acidities and lacked flavor, whereas cheese having higher fat acidities commonly had more flavor. Addition of pancreatin to pasteurized milk for cheese resulted in a disagreeable rancid flavor immediately after manufacture and the condition did not disappear during ripening; often the ripened cheese was bitter. Desiccated mammary tissues, or extracts of them, were lipolytic and their addition to pasteurized milk commonly had a desirable effect on the cheese made from it. Early in the ripening the tissues or extracts, particularly in the larger amounts, commonly caused rancidity in the cheese but this disappeared as the ripening continued and after some time the flavor generally was better than the flavor of cheese made from pasteurized milk without the addition. The mammary tissues or extracts increased the fat acidity of the cheese.

Babel and Hammer [93] found in trials with pasteurized milk that use of rennet paste resulted in an increase in fat acidity of the cheese. Some of the cheese showed rancidity early in the ripening but this disappeared, or nearly disappeared, when the cheese was 6 mo old. Cheese that was not rancid was superior in flavor to the control cheese. Use of a small amount of rennet paste tended to mask the acid flavor in the control cheese. Mulberry juice is actively lipolytic. When it was added to pasteurized milk it did not improve the flavor of the cheese; small amounts had little effect and larger amounts resulted in rancid and unclean flavors.

The general evidence indicates that fat hydrolysis has an influence on the flavor of cheese and that one of the important differences between raw-milk and pasteurized-milk cheese is the absence of lipase action in the latter. It appears that in ripening of cheese made from raw milk the products of lipolysis that would give a rancid flavor if they accumulated are destroyed and that the mechanism even may be active enough to cause disappearance of a rancid flavor after it once is evident, provided it is not too conspicuous. The trials in which lipase was added to pasteurized milk for cheese have not yielded the desired results. Presumably, the enzyme preparations caused changes that were not satisfactory from the standpoint of flavor development, their action being different from that of milk lipase.

SWISS OR EMMENTHALER CHEESE

Swiss, or emmenthaler, cheese is a typical hard variety in which normal ripening apparently involves the action of various bacteria, with formation of eyes and a sweet flavor. Experience and laboratory investigations have suggested that one or more types of organisms be added to the milk to be used for swiss cheese. These include (1) *S. thermophilus* to improve the general quality, (2) a *Lactobacillus* to limit abnormal fermentation and aid in controlling the ripening, and (3) a propionic acid organism (genus *Propionibacterium*) to aid in development of the desired flavor and, perhaps, eye formation. The various cultures are propagated in milk, whey, etc. From the standpoint of its manufacture, swiss cheese is characterized by a relatively high cooking temperature, such as 52.2 to 60 C (126 to 140 F), which also means that the cheese on the press remains at a high temperature for an extended period; this greatly influences the flora of the cheese. In some cases cheese of a swiss type is made with much lower cooking temperatures.

The characteristic organisms of swiss cheese are the propionic acid bacteria. Presence of these organisms is so characteristic of swiss cheese that they distinguish it from other hard cheeses.[94]

Importance of milk quality. The relationship of the condition of the milk to the quality of swiss cheese has long been recognized, and twice-a-day delivery of milk has been adopted extensively in the industry. Rogers *et al.*[95] noted that one of the most important factors influencing the quality of swiss cheese is the bacteriological condition of the milk. If the methylene blue reduction time was not over 3 hr, the chances were one in three that the cheese would be grade A or B, whereas if the time was over 3 hr, the chances increased to two in three. The chances of making a grade A or B cheese could be increased to three out of four by adjusting the cultures and the making process to give a *p*H at dipping above 6.35 and not over 6.51. If the *p*H at this time was over 6.51, there was only one chance in eight or ten that the cheese would be grade A or B. The authors suggested that a relatively low *p*H at dipping may not be responsible for inferior quality in cheese but that conditions producing rapid acid development may promote growth of abnormal bacteria. Evidently, certain bacteria may have a deleterious effect, even when their activity in the kettle is limited. When the *p*H is unusually low, defective cheese may be due to the abnormally high acid.

Clarification of milk. On the basis of general observations and experience, clarification of the milk has been widely adopted by the swiss-cheese factories of the United States. Matheson *et al.*[96] investigated the intermediate factors in the improvement of the cheese. Specific effects of clarification of the milk included: (1) decrease in the tendency of the fat to form aggregates on standing, (2) removal of a large proportion of the leucocytes from mastitis milk, (3) an increase in the rate of multiplication of culture organisms and improvement in results of fermentation tests, (4) increase in concentration of oxygen and decrease in carbon dioxide, (5) increase in rate at which the oxidation-reduction potential changes at 30 C (86 F), and (6) slight decrease in stability to alcohol. Effects of the process on the properties of the cheese included: (1) marked decrease in number and increase in size and uniformity of eyes, (2) increase in firmness of the cheese and in incidence of the glaesler defect, (3) increase in the rate of multiplication of culture organisms and of acid formation, (4) decrease in moisture content and yield of cheese, and (5) increase in fat loss in the whey. Clarifying the milk at a relatively low temperature with a relatively slow bowl speed diminished the effects of clarification on the milk and on the cheese, whereas decreasing the rate of flow through the bowl by one-half and increasing the temperature from 21 to 32 C (69.8 to 89.6 F) increased the effects. The beneficial effects of clarification were especially pronounced with mastitis milk.

General morphologic groups of bacteria. Eldredge and Rogers[97] studied the changes in the general morphologic groups of bacteria during ripening of swiss cheese by plating on a beef extract peptone whey agar and, with each plating, picking all the colonies from a representative plate. More than 900 cultures were studied in detail. Fermentation tests failed to divide them into distinct groups but they were sharply differentiated morphologically into cocci, short rods, and long rods. Young cheese contained short rods almost exclusively, but these decreased slowly while the long rods increased. After 6 to 8 weeks the two groups were present in about equal numbers, and at the end of the ripening long rods made up most of the flora. The cocci appeared in small numbers at intervals in the earlier stages of ripening. The rods included typical lactobacilli but there were all gradations from these to cultures producing no acid in milk.

***S. thermophilus* in cheese.** Because of the high cooking temperature used with typical swiss cheese, organisms that are relatively heat resistant are of special importance. Frazier *et al.*[98] found that under most conditions use of cultures containing *S. thermophilus* improved the quality of cheese, the improvement being especially marked in eye formation, texture, and flavor; many of the cheese made with *S. thermophilus* showed a tendency to checking. Pure cultures of *S. thermophilus* were as satisfactory as kettle-whey cultures and avoided the uncertainties of the whey cultures. Frazier *et al.*[99] noted that *S. thermophilus* usually increased during the whole process in the cheese kettle, but the increase commonly was more rapid late in the process when the temperature was high; *S. lactis* increased early but was stopped by the high cooking temperature. Later, Frazier *et al.*[100] reported that *S. thermophilus* usually began to grow within 3 or 4 hr after dipping and increased rapidly until the sixth or eighth hour, after which the increase was slow or there was a gradual decrease to the twenty-first hour. *S. thermophilus* appeared to be responsible for the rapid decrease in *p*H of the cheese during the first 7 or 8 hr; most of the cells were very large which may explain the great fermentative activity per cell. Burkey *et al.*[101] considered the essential function of *S. thermophilus* in swiss cheese on the press to be production of acid that facilitates drainage from the interior of the cheese during the first 4 to 6 hr after dipping. In studies on the use of *S. thermophilus* in ripening milk for swiss cheese, Frazier *et al.*[102] noted that such ripening usually improved the quality of the cheese when the methylene blue reduction time of the milk was 5 to 6 hr, or longer, but not when the reduction time was shorter.

***Lactobacillus* types.** The *Lactobacillus* cultures used in swiss cheese apparently have been of different types, including *L. bulgaricus, L. helveticus, L. casei,* and unidentified species. Apparently, the lactobacilli do not increase in the kettle and usually have decreased by the time of dipping, whereas on the press[100] they decrease for a time and then increase fairly rapidly. This relationship suggests that the organisms are of primary importance during the ripening process when, presumably, they aid in the protein breakdown.

Activity of culture organisms. Activity of the culture organisms used is very important; also their heat resistance during the cooking is significant.

Peppler and Frazier[103] found that active cultures of *S. thermophilus* and *L. helveticus* could be prepared with considerable latitude in incubation time and temperature, and also that variations within limits in methods of incubation of stock cultures could be employed, with assurance that satisfactory growth and fermentation of the cultures will be maintained. They[104] noted that a variety of modifications, depending on incubation and storage temperature and the culture medium employed, can be applied to methods of handling cultures of thermoduric bacteria without lessening the activity and heat resistance. By using a better culture medium, one with added accessory substances, the heat resistance of cultures is not only assured but actually increased. Results indicate cultures of great heat resistance can be produced by improving the culture medium and/or by incubation at 40 C (104 F) instead of 37 C (98.6 F). In the case of *S. thermophilus,* growth at 40 C requires a medium of highest quality and proper control of the incubation period to provide cultures of proper maturity. Mixed cultures of the film yeast *Candida krusei* and *L. helveticus* exhibited greater heat resistance than pure cultures of the latter species; frequent transfer of the mixed cultures at 37 C reduced the influence of *Can. krusei. S. thermophilus* grown with *Can. krusei* was more active after heating than pure cultures of the *Streptococcus;* successive transfers of the mixed cultures did not decrease the influence of *Can. krusei.* After symbiosis[105] with *Can. krusei, S. thermophilus* and *L. helveticus* from areas near the surface film were more active following severe heat treatment than bacteria from the same culture but at lower levels. Influence of the film yeast extended some distance below the active growing region. Filtrates of cultures of *Can. krusei* contained substances that stimulated acid production by *S. thermophilus.* Mature bacterial cultures stored under conditions in which the apparent actions of the film yeast were simulated by reduction in acidity, lowering of oxygen tension, and addition of neopeptone showed that each treatment alone produced no consistent difference in activity but that the combination of the three markedly increased the heat resistance of *S. thermophilus.*

Eye formation. Eyes of the proper size and distribution are very important in swiss cheese. Normal eyes vary in diameter from about 0.5 to 1.25 in. and are so spaced that there is 1 to 3 in. between them. Under abnormal conditions the eyes may be small and numerous, or large and few in number, while in some instances eye formation fails. Ability of various bacteria to produce gas suggests that eye formation is due to bacterial action.

Clark [106] found that the gases of normal eyes of swiss cheese are carbon dioxide and nitrogen; the nitrogen is from air occluded in the curd at the time of making so the carbon dioxide is the only gas of significance. In some instances he noted an evolution of gas characterized by the presence of hydrogen and considered it to be due to formation of gas from sugar; this type of gas production occurs soon after the cheese is made and is detrimental.

Because of the uneven distribution of bacteria in cheese it might be assumed that the eyes form at the location of colonies of the gas-producing organisms. However, the comparatively few eyes in a normal swiss cheese make this improbable and, moreover, the types of organisms present near the eyes appear to be the same as those at a distance. Clark [107] pointed out there is no need to assume that the gas separates as a bubble at the point where it is produced, and showed that in agar or gelatin gas diffuses considerable distances. He increased the size of a gas bubble blown in agar by growing gas-producing organisms some distance from it; in certain of the trials the gas diffused through a collodion sac in its passage from the region of bacterial growth to the point where it appeared as a bubble. Cultures of gas-forming organisms in agar or gelatin containing a fermentable material commonly illustrate this gas diffusion; bubbles are not formed around each colony, and colonies often are found at considerable distances from bubbles. Clark concluded that the gas in swiss cheese separates at points that have no necessary relation to the localities where the gas is produced. When the curd particles were stained with congo red by addition of this dye to the kettle just before the curd was drawn, the interior walls of normal eyes were stained, which led to the assumption that the eyes developed between curd grains.

Propionic acid organisms. Von Freudenreich and Orla-Jensen [108] isolated propionic acid bacteria, which they considered the cause of eye formation in swiss cheese. The essential organism fermented lactates with production of propionic acid, acetic acid, and carbon dioxide. From trials involving addition of dilutions of cheese to calcium lactate broth, the investigators concluded that the organisms occur in cheese in numbers from 10,000 to 200,000 per gram.

Sherman [109] isolated a propionic acid organism from swiss cheese and considered it to be the essential organism for production of eyes and the characteristic flavor. It fermented lactates with the production of carbon dioxide and volatile acids, especially propionic and acetic acids; lactose also yielded carbon dioxide and volatile acids. Using a sodium lactate broth containing dried yeast, this investigator found that all of 16 samples of domestic swiss cheese, which varied in flavor from excellent to very poor but each of which showed eyes, contained a lactate-fermenting organism in numbers exceeding 1 million per gram; the organism was recognized through its ability to produce volatile acid in the medium employed. Sherman concluded that he found the organism in large numbers because the medium used was very favorable. The organism was easily isolated by adding dilutions of cheese to agar made with peptone, dried yeast, and sodium lactate and then sealing the agar in glass tubes.

The characteristic change brought about by the propionic acid organisms is the breakdown of lactic acid (or lactate) according to the reaction,

$$3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O.$$

Shaw and Sherman [110] found that in addition to fermenting lactose and lactic acid the organism studied by them produced carbon dioxide and propionic and acetic acids from succinates, glycerol, peptone, and perhaps to a slight degree from butter fat; peptone gave a greater proportion of acetic to propionic acid than the other substances attacked. Aspartic acid was shown to serve as a source of carbon dioxide and acetic acid. Considering the various materials from which the propionic acid organisms can produce carbon dioxide and volatile acids, growth should be greatly influenced by the activity of other species in making such materials available. Sherman and Shaw [111] found that the propionic acid production in a lactose peptone broth containing calcium carbonate was increased by various species, including *S. lactis* and *L. casei*.

Apparently, there are certain differences between the propionic acid organisms that have been considered of importance in swiss cheese. *Propionibacterium shermanii* is a species that has been widely used in making cheese. It appears this organism does not increase in the kettle [99] or on the press,[100] so that its activity is confined to the ripening period. Demeter and Janoschek [94] found that in swiss cheese propionic acid organisms increase rapidly after salting until in about 70 days they outnumber the lactic acid bacteria; later, they decrease, as do the other bacteria, but remain in the majority. Propionic acid organisms were found in other hard and semi-hard cheeses but did not exceed the lactic acid bacteria in number.

The propionic acid organisms generally are considered to influence the flavor of cheese but certain investigators now believe that they have little to do with eye formation, although at one time they were considered of primary importance in this connection. Cheese that lacks eyes may have a satisfactory flavor, and cheese that has eyes may lack flavor, so the relationship between eye formation and flavor production is not a close one. The balance between production and diffusion of gas appears to be the important consideration, rather than the particular species producing the gas.

The propionic acid organisms are readily enumerated and isolated, using a lactate agar in shake cultures; the colonies produced are winged rather characteristically. A satisfactory composition for lactate agar is: [86]

	Per Cent
Peptone	2.0
Sodium lactate	1.0
Yeast extract	1.0
Washed agar	1.5
Water to make	100.0
*p*H 7.0	

The organisms can be tentatively identified on the basis of staining reactions and production of catalase. The final step in identification is to determine their ability to ferment lactic acid, or lactate, under anaerobic conditions with the formation of propionic and acetic acids, the ratio ordinarily being approximately 2:1. By using a special yeast lactate agar, Demeter and Janoschek [94] lowered the oxidation-reduction potential with sodium sulfite so that with an overlayer of the medium and a layer of sterile paraffin oil, the organisms could be grown in petri dishes.

Flavor from propionates. The propionates, of which calcium propionate presumably is the most significant, are important flavor contributors of swiss-type cheese. The salts have a sweet and somewhat bitter flavor. Babel and Hammer [112] found that addition of calcium or sodium propionate to process swiss-type cheese lacking in flavor gave the product a sweet flavor. Swiss-type cheese with considerable characteristic sweet flavor yielded a much higher volatile acidity on steam distillation than cheese lacking in flavor.

Protein decomposition in swiss cheese. The general agents responsible for protein decomposition in swiss cheese are lactic acid organisms and enzymes, much as in cheddar cheese, although the relationship between them may vary from that in cheddar cheese. Watson [113] noted that production of a desirable soft texture in swiss cheese was not necessarily coincident with the highest degree of proteolysis, and that variations in the colloidal structure of cheese, as shown by the difference in binding of the water, exert an important effect on the texture.

DEFECTS IN SWISS CHEESE DUE TO ORGANISMS

Lack of eye formation. Swiss cheese sometimes fails to develop eyes in the normal manner. Presumably, this is due to an irregularity in the ratio between production and diffusion of gas.

Gassy cheese. In some instances excessive amounts of gas are produced, and instead of the desired eye development the cheese shows many gas holes; often the shape of the cheese becomes very abnormal. Gas formation in swiss cheese may cause serious economic losses; it sometimes occurs soon after the cheese is made, but in other cases it is delayed. The responsible organisms are of various types.

Russell and Hastings [114] investigated an outbreak in a Wisconsin factory and found a lactose-fermenting yeast responsible. When the organism was added to milk that was made into cheese it caused an extremely gassy condition, and the cheese was decidedly lacking in flavor. The investigators noted certain practices, at one time common, that tend to favor growth of the organism: (1) holding the whey so that the butter fat can be skimmed off, (2) use of whole rennets soaked in sour whey, and (3) use of individual barrels, which are rarely cleaned, to hold whey for the farms.

Burri [115] reported that an excessive number of large holes and a splitting of the rind, together with an objectionable flavor, were due to large numbers of butyric acid organisms that came from silage. Beginning and ending of the abnormality in the cheese coincided so definitely with beginning and ending of the silage feeding that the relationship was very clear.

Albus[116] investigated the cause of unusually large numbers of gassy cheese at a factory and, after failing to isolate a gas-forming organism that would grow aerobically, obtained a strain of *Clostridium welchii* that was responsible for the abnormal condition. Experimental cheese showed both the niszler type of gassy fermentation, which is characterized by a uniform distribution of very small holes that are detected when the cheese has been made for some time, and the pressler type, in which the gassy condition is noted on the press. In a few instances the cheese made from milk inoculated with the culture of *Cl. welchii* showed little or no gas, although the method of manufacture was normal. The results suggest that some undetermined factor or group of factors played an important part in the development of the gassy condition.

Weiser[117] reported that examination of gassy or split-rind swiss cheese showed two types of organisms, anaerobic bacteria (*Clostridium perfringens*) and lactose-fermenting yeasts (*T. cremoris*). The organisms were conspicuously absent in normal cheese. In extremely gassy cheese both species were present but in mildly defective cheese the latter type was not found. When the farms employed insanitary practices the fresh milk showed the presence of both types.

Abnormal flavors and aromas. Swiss cheese sometimes develops abnormal flavors and these may suggest an objectionable protein decomposition. Factories have occasionally experienced an undesirable fermentation in which the cheeses are referred to as "stinkers" because of the foul odor. The defect may be confined to small areas within the cheese so that it cannot be detected from the outside; these areas may be dark in color. Hastings and Frazier[118] found that in many cases as the odor increased it was characterized by hydrogen sulfide. Large numbers of butyric acid organisms were found in the spoiled areas but very few were in the unaffected cheese.

According to Dorner and Thöni,[119] odoriferous fermentation or gray decay of swiss cheese appears sometime after the cheese is made. It is first evident as the *p*H of the cheese rises and at the end of a few weeks the cheese shows a putrid odor and grayish color. The causative organism is *Bacterium proteolyticum.* When the milk used for the cheese contained as many as 10 of the organisms per milliliter secondary odoriferous fermentation ensued. With heavy infection the cheese shows a high *p*H, a bad odor, gray or dark spots, and gas. Burri[115] reported an objectionable flavor associated with the development of a white, crumbly condition and large, irregular holes in parts of the cheese; *Bacillus putrificus* was found in this material.

Swiss-type cheese. A swiss-type cheese, prepared in a vat from pasteurized milk with relatively low cooking temperatures, has been made in various countries, including the United States. Babel and Hammer [86] found that certain propionic acid cultures rather regularly produced excellent or good flavors in this type of cheese but none of the cultures was consistent in producing good eye formation; control cheese in which no propionic acid organisms could be detected in 0.1 gm sometimes developed satisfactory eyes. By increasing the number of propionic acid bacteria added to the milk there was a corresponding increase in the degree of sweet flavor produced; with high inoculations the cheese sometimes was criticized as too sweet. When good flavor developed in the control cheese, propionic acid organisms were present in large numbers. Delaying the salting of the cheese resulted in an increase in the numbers of propionic acid organisms and in the sweet flavor but the cheese tended to flatten. Addition of propionic acid organisms to the milk gave cheese with a higher volatile acidity and a higher acid number on the fat; the degree of sweet flavor was rather directly related to the amount of volatile acid present. *p*H values on the cheese at 90 days were not definitely correlated with the quality. Cheese containing large numbers of *Escherichia-Aerobacter* organisms had numerous ragged eyes, which were more objectionable than the flavor; with large numbers of anaerobic spore formers present, the flavor of butyric acid increased with the age of the cheese.

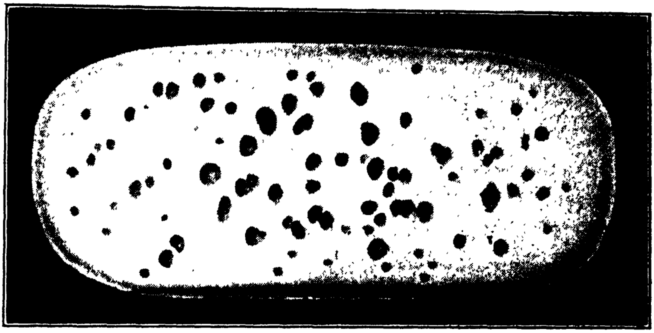

FIG. 45. Small size swiss-type cheese showing desirable eye formation. Iowa Agr. Exp. Sta. Res. Bul. 264.

BRICK CHEESE

Brick cheese is a semi-hard, bacteria-ripened variety which presumably originated in the United States. Commonly, the body is softer than that of cheddar but not so soft as that of a typical soft cheese. A surface growth of organisms—the smear—develops on the cheese and appears to be essential for production of the typical flavor.

Lactic acid streptococci. The culture organisms added in the making of brick cheese produce lactic acid rapidly during the manufacture. Garey *et al.*[120] found that in a typical trial in which 0.6 per cent of a milk culture of *S. lactis* was used in making the cheese, direct counts indicated a gradual increase in numbers of the organism, and there was a small decrease in *p*H between dipping and the sixth hour. Between the sixth and the twelfth hours, the numbers increased rapidly; after the twelfth hour the numbers increased slowly, with the maximum being reached between the third and the sixth days. Cooking to 44.4 C (112 F), in comparison with 41.1 C (106 F), was harmful to the later growth and activity of *S. lactis. S. thermophilus* never attained appreciable numbers in the cheese. In a typical trial using 0.6 per cent of a milk culture of *S. thermophilus* in making the cheese, the organism multiplied rapidly in the vat and after dipping increased steadily until the sixth hour. Between the sixth and the twelfth hours, the numbers increased more slowly and reached their maximum. Cooking to 44.4 C favored growth and activity of *S. thermophilus.* Late growth of *S. lactis* occurred. When equal proportions of milk cultures of the two organisms were used in making the cheese, *S. thermophilus* increased rapidly in the vat and reached its maximum the third hour after dipping. At this time *S. lactis* was just beginning to multiply; it increased rapidly and reached its maximum 12 hr after dipping. Growth of the culture organisms was slower in cheese from pasteurized milk than in cheese from raw milk.

Rapid production of acid during manufacture of brick cheese is necessary and is dependent on the culture organisms added to the milk. Langhus *et al.*[121] noted that the quality of brick cheese usually is no better than the quality of the culture employed.

Foster *et al.*[122] isolated 1,016 cultures from 18 lots of brick cheese, at different stages of ripening, and found that *S. lactis* was the predominant organism throughout the ripening of cheese in which it was used as part or all of the culture. It increased to 1 to 3 billion per gram during the first 1 to 4 days and declined after 3 to 4 weeks. It also increased slowly in cheese in which it had not been used as a culture and reached several hundred million per gram after 1 to 2 weeks. When *S. thermophilus* was used as the culture, it increased rapidly to 1 to 2 billion per gram at 12 hr, then decreased rapidly after 1 to 2 weeks, and seldom was found later in the ripening.

Other organisms in cheese. Lactobacilli were found in raw-milk cheese after 1 or 2 weeks by Foster *et al.*[122] but they seldom appeared in pasteurized-milk cheese. *L. casei* was encountered most frequently and in numbers up to 10 to 100 million per gram. It was considered the most important organism in bringing about changes in body during the ripening of the cheese and also appeared to contribute to the flavor.

Salting. Byers and Price[123] found that salt penetrated the outer layer of brick cheese quickly, but 8 weeks of curing elapsed before the salt content was practically uniform throughout the cheese. Dry salting was effective but not so uniform as brine salting. Desirable results were obtained when the cheese took up salt to the extent of about 2 per cent of the final weight. Excessive salt caused hard, curdy body, unnatural white color, slow curing, loss of yield, and delayed lactose fermentation, whereas low salt encouraged abnormal fermentations and caused weak body and open texture.

Smear of cheese. Langhus *et al.*[124] reported that the smear on brick cheese appears only when growth of several types of organisms occurs. Yeast-like organisms develop first and are followed by micrococci and rods of the *Bacterium linens* type. The smear reduces the acidity of the surface layer of the cheese, the decrease being greater when the smear development is luxuriant. The surface sections of brick cheese were the same in water- and alcohol-soluble nitrogenous compounds as the inner sections, which suggests there is no ripening from the outside. However, gradual diffusion of the characteristic surface flavor was obvious. The authors suggest that the slight odor of extensive protein decomposition in brick cheese is produced by the surface organisms and that the characteristic odor is absorbed by the cheese. Curing brick cheese at 14.4 to 15.6 C (58 to 60 F) under conditions that favored activity of the smear gave the cheese more flavor and caused a more rapid protein breakdown, but as measured by the analytical procedures the latter occurred uniformly throughout the cheese.

Bacterium linens. *Bact. linens* is a typical cheese organism in that it grows on the surface of various semi-hard and soft cheeses and apparently plays a role in the protein breakdown and flavor development. Albert *et al.*[125] noted that in milk it produced an alkaline reaction and then conspicuous proteolysis; amino nitrogen was significantly increased. The organism readily tolerated 15 per cent sodium chloride in broth or skim milk and there was some evidence of growth in considerably higher concentrations. The salt tolerance enables it to grow on the surfaces of various cheeses. It grew at *p*H values of about 6.0 and about 9.8 but not at about 5.0. Corresponding volatile acids were produced from various lower alcohols, for example, acetic acid from ethyl alcohol. Catalase was actively produced. The organism was not lipolytic. *Bact. linens* was found widely distributed in certain dairy products and in materials about stables, especially feed, hay, straw, water, and manure. It also was found in the mouths of cows and in the air but rarely in soil. A special technic, involving use of cheese agar with 5 per cent salt and incubation of the plates in oxygen, facilitates its isolation.

Defects. According to Price and Spicer,[126] the *p*H of cheese 3 days after making is a useful index of the acid characteristics of the ripened cheese; it should not be below 5.1.

Garey *et al.*[120] found that when *S. lactis* alone was used as the culture, control of the moisture at dipping was necessary to avoid sour flavor and weak or crumbly body; if the moisture content at 2 days was above 42 per cent, the defects appeared. With *S. thermophilus* alone as the culture, fermented flavor and open texture developed in the cheese. A combination of *S. lactis* and *S. thermophilus* as the culture gave a better cheese than either organism alone. Foster *et al.*[127] reported that when the moisture content of the cheese remained above 39 per cent, fermentation of all the lactose lowered the *p*H to about 4.8 and acid flavor and short crumbly body resulted. In an attempt to prevent development of excessive acidity, they used a washing procedure to remove some of the lactose from the curd before dipping. After this treatment enough fermentable lactose remained in the curd to lower the *p*H to about 5.00 to 5.15 in cheese with a moisture content of 40 to 42 per cent. A mild wash, in which 25 to 28 lb of water per 100 lb of milk was added to the curd plus whey in the vat and then 50 to 55 lb of whey was removed and replaced by an equal volume of water at 32.2 C (90 F), gave the best results, the cheese having a mild clean flavor, soft smooth body, and medium close texture. Undesirable fermentations were more common in washed curd than in conventional cheese, presumably because of the lower acidity in the former. With good-quality milk and a mixed culture (*S. lactis* and *S. thermophilus*), undesirable fermentations did not appear and the washed curd cheese was superior in flavor, body, and moisture content.

Hanson *et al.*[128] found that lack of acid development, low salt content, and large loaves of cheese tend to encourage development of late gas in brick cheese but that these are not necessarily the only factors involved in the defect. In trials by Foster *et al.*[122] late gas formation by anaerobic spore-forming bacteria occurred whenever the *p*H did not drop below 5.3 during the first 3 days of ripening. The defect was most pronounced in cheese made with *S. thermophilus* from pasteurized milk by the washed curd method.

Bitterness in brick cheese has been attributed to excessive numbers of *S. liquefaciens.*[122]

BLUE-VEINED CHEESES

The blue-veined cheeses are semi-hard, mold-ripened varieties in which the characteristic mold normally develops throughout the cheese mass as the cheeses cure and produces blue or bluish-green veins. The mold is active in bringing about changes in flavor and texture. Representatives of this group of cheeses are the French roquefort, which is made from sheep's milk, and the Italian gorgonzola and the English stilton, which are made from cows' milk; various countries, including the United States, produce blue cheese from cows' milk.

Milk used. Commonly, the blue-veined cheeses are made from raw milk because fat hydrolysis is an important part of the ripening and milk lipase aids in it. With poor-quality milk, bacterial defects may appear in the cheese; these can be largely controlled by pasteurization but ripening then is delayed since, with the milk lipase destroyed, all the fat hydrolysis must be brought about by the mold.

***S. lactis* in blue cheese.** In the making of blue cheese, acid development occurs through activity of *S. lactis.* Its presence in satisfactory numbers is assured by addition of lactic acid culture to the milk. If acid does not develop properly the cheese fails to drain normally and growth of objectionable bacteria may occur. After reaching large numbers, *S. lactis* soon dies. Evans[129] found that it disappeared from cheese in about 2 or 3 weeks, and ascribed this rapid destruction to the high concentration of salt. However, products of fat hydrolysis in the cheese may be a factor.

In trials by Coulter *et al.*,[130] blue cheese developed a *p*H of about 4.7 within 24 hr of manufacture. Except for a temporary drop following piercing, the *p*H gradually increased to about 6.5 after about 3 mo and then gradually decreased to about 5.7 after 9 mo.

In some instances the *p*H of blue cheese reaches relatively high values, presumably when the normal mold develops extensively, and such values often are accompanied by darkening of the cheese and undesirable flavors.

Bacteria other than *S. lactis*. A number of bacterial species other than *S. lactis* have been found in blue cheese by various investigators. Thom *et al.*[131] reported the presence of lactobacilli, liquefying bacteria, and yeasts, whereas Evans [129] found lactobacilli and streptococci. In the stilton cheese investigated by Percival and Mason [132] a spore former regularly was present but never abundant.

It appears that *S. lactis* is the only necessary bacterial species in blue cheese. Other organisms often are present, because of their presence in milk and their ability to survive conditions in the cheese; although they may develop actively, changes that they bring about are not vital to normal ripening.

The mold of blue cheese. The typical mold of blue cheese was named *Penicillium roqueforti* by Thom.[133] In some of the older literature the mold in blue cheese is referred to as *Penicillium glaucum* [134] but it is probable that more than one species has been included under this name. *P. roqueforti* is by no means limited to blue cheese, and frequently is found in other dairy products. It has been isolated from silage and undoubtedly is more or less regularly present in the stable surroundings.

In the making of blue cheese, mold spores, in the form of a powder, are mixed with the curd just before it is put into the hoops or are sprinkled on the curd in the hoops. The powder commonly is prepared by growing the mold on bread or other material and then drying and grinding the portions showing heavy mold development. Hussong and Hammer [135] sterilized small cubes of whole wheat bread in widemouth bottles and then inoculated and incubated them. After a heavy growth was obtained the bread was dried and ground. Powders prepared in this way contained 30 million to 1.5 billion spores per gram, as determined by plating. When the spores were suspended in water and counted directly, the values were much higher; many of the spores were in groups.

After the cheese has drained properly it is salted, by either brine or dry salting, and then punched to admit air to the interior of the cheese. Both of these procedures have a definite effect on the growth of molds in the cheese.

The dominance of *P. roqueforti* in blue cheese, when spores and vegetative growth of a variety of molds are present as a result of contamination during production and handling, presents a problem in the relationship of this organism to the environment. Thom and Currie [130] analyzed the gas in both imported and experimentally made cheese and found the percentage of free oxygen low, 7.00 per cent being the highest value in five analyses. The carbon dioxide varied from 21.14 to 40.95 per cent; the percentages appeared to be highest in comparatively young cheese and to fall off through diffusion as the cheese aged. The investigators studied a number of species of *Penicillium* and *Aspergillus* and noted that *P. roqueforti* tolerated a high percentage of carbon dioxide and a low percentage of oxygen better than any of them. However, *O. lactis* also grows at a low oxygen tension. Thom *et al.*[131] found this species would develop in blue cheese when the salt concentration was 2 per cent, but all abnormalities due to it disappeared with 4 per cent salt in the newly made cheese. Accordingly, it appears that mold growth in blue cheese is largely limited to *P. roqueforti* because it tolerates both low oxygen tension and high concentration of salt and other molds do not.

FIG. 46. Mold growth in blue cheese. Courtesy F. J. Babel.

Certain lots of blue cheese show areas of mold growth that definitely are not due to *P. roqueforti*. In many cases these represent invasion from the surface of the cheese, but occasionally they appear to originate in the interior. Irregularities in composition of the cheese may be responsible for most of the areas, but some of them appear to develop in cheese that is quite normal.

Changes produced by *P. roqueforti*. The proteolytic action of *P. roqueforti* is evident from the appearance of milk cultures. When a blue cheese is not punched, failure of the mold growth, due to the lack of air, quickly is reflected in the body of the cheese. Instead of the curd breaking down normally it remains tough and crumbly. This suggests that *P. roqueforti* is the principal proteolytic agent in blue cheese. Currie[137] reported data (obtained by Dox) on the nitrogen distribution in ripened roquefort cheese; 45.5 per cent of the nitrogen was in insoluble forms, 10.7 per cent as caseoses, 8.6 per cent as peptones, 29.1 per cent as amino acids, and 6.1 per cent as ammonia. The high percentages of insoluble nitrogen and amino acids and the comparatively low percentages of caseoses and peptones are characteristic of hard and some semi-hard cheeses.

Fat hydrolysis during ripening of blue cheese was studied in detail by Currie,[137] who pointed out that it is due chiefly to a water-soluble lipase produced by the mold. The hydrolysis eventually results in the accumulation, in both free and combined forms, of the acids present in milk fat; of these, caproic, caprylic, and capric acids and their readily hydrolyzable salts are especially important. Currie grew the mold on media to which butter fat had been added and found, from the acid number, that more than half the fat had been hydrolyzed. Ethyl acetate, ethyl butyrate, triacetin, and tributyrin also were hydrolyzed by a preparation made by triturating air-dried mycelium with powdered glass. Solubility of the lipase in water is of special interest in that it explains the fat hydrolysis in parts of the cheese where there is no mold growth.

Thiobodeau and Macy[138] found that, on a 1 per cent casein substrate and with phosphate buffer used, the protease of *P. roqueforti* reached its optimum activity between *p*H 5.8 and 6.3. The mold seemed to produce only one protease which appeared to be of the trypsin type but different from animal trypsin in that its optimum activity occurred at a lower *p*H and over a wider *p*H range. Optimum activity of the lipase of *P. roqueforti* was favored over a wide *p*H range, between 5.3 and 7.5 when the substrate was 3 per cent butter oil emulsion in the presence of acetate buffer. Concentrations of sodium chloride such as exist in blue cheese did not retard action of the protease or lipase.

P. roqueforti, like many molds, is favored in its growth by organic acids that, presumably, supply a satisfactory carbon source and are used for the energy supplied. Lactic acid is of importance in this connection. It appears that some of the acids liberated from the fat also are used by the mold. Bryant[139] noted that when volatile fatty acids were added to milk inoculated with *P. roqueforti* there was a destruction of the acids. Reduction was largest with butyric acid and became less as the molecular weight increased. It is probable that during the early stages of the ripening of blue cheese the lower fatty acids liberated are used by the mold, and that when conditions become less favorable for the mold the acids tend to accumulate.

Hammer and Bryant[140] found that cultures of *P. roqueforti* in milk containing caprylic acid developed an odor suggestive of blue cheese. The compound responsible was identified as methyl-*n*-amyl ketone. It appears to be formed from caprylic acid through action of *P. roqueforti* under unfavorable growth conditions as a result of a beta oxidation and elimination of carbon dioxide from the carboxyl group. Heitzman[141] noted that non-proliferating mycelium brought about the same type of change in caproic acid with formation of methylpropyl ketone. Stokoe[142] concluded that various methyl ketones may be formed by molds because absorption of poisonous fatty acids by the mycelium impedes respiration.

Characteristic flavor of blue cheese. Currie[137] pointed out that the flavor of blue cheese is due largely to the free and combined forms of caproic, caprylic, and capric acids liberated from the milk fat. These compounds have a sharp, peppery taste and are responsible for the characteristic effect when the cheese is taken into the mouth.

Since fats of the milk of various species of animals differ in composition, a difference in suitability of milk from the various species for the manufacture of blue cheese might be expected. Currie investigated this from the standpoint of the use of cows' milk and concluded that differences between the fats from typical roquefort cheese and from cows' milk are not great enough to necessitate the exclusive use of sheep's milk in the manufacture of this type of cheese; however, cheese made from sheep's milk may be expected to have more of a peppery taste than that from cows' milk.

The odor of methyl-*n*-amyl ketone is rather definite in many lots of blue cheese and appears to be a significant flavor contributant. In some lots it is not evident, although it may be present to some extent; occasionally, it is so pronounced it is objectionable. Stärkle[143] distilled roquefort cheese and obtained about two drops of material that had an intensive odor of methyl-*n*-amyl and methyl-*n*-heptyl ketones. The amount was too small to permit separation and identification. Methyl-*n*-heptyl ketone apparently is formed from capric acid.

Slime on blue cheese. During ripening of blue cheese a slime that commonly includes various species of bacteria, yeasts, and molds develops on the surface. Thom *et al.*[131] eliminated the slime organisms completely enough by paraffining to indicate that they have only a secondary function in the ripening. Evans,[129] who found the slime to be made up of characteristic types of micrococci, rod forms, and yeasts, concluded that enzymes from the slime do not appear to be essential to ripening.

The sequence of organisms on the surface of blue cheese has been reported[144] as follows: Before salting large numbers of *S. lactis* were present but after salting they largely disappeared and were replaced by yeasts. As the yeasts increased the *p*H rose from 4.8 to 5.0. At 3 weeks the *p*H was 5.9 and the yeasts began to disappear and were replaced by rods. At 6 weeks the *p*H was 7.3, red color was found in the slime, and yeasts, rods, and cocci as well as *P. roqueforti* were present. In the interior of the cheese the *p*H was 4.8 at 1 day and changed little during 6 weeks.

Normal slime formation often is an index to a satisfactory humidity in the curing room. Its removal, by washing or scraping, before wrapping of the cheese and perhaps during the curing involves both labor and a reduction in weight of the cheese so that certain manufacturers of blue cheese have attempted to limit slime formation by partially covering the surface with parchment or waxed paper or by dipping the cheese in wax.

Species of molds in blue cheeses. It often has been assumed that *P. roqueforti* is the mold regularly associated with all blue-veined cheeses. However, Steuart[145] found that, in 12 such cheeses of different varieties, this organism was thoroughly dominant in 8, was present in another, and was apparently absent in 3. Golding[146] noted that the dominant mold in wensleydale cheese is not identical with *P. roqueforti.* Satisfactory blue cheese has been made by inoculating the curd with *Penicillium oxalicum.* Lane[147] compared eight strains of penicillia from the standpoint of the volatile acid and flavor produced in blue cheese. Certain strains formed more volatile acid than others and certain strains were regularly associated with cheese having a desirable flavor; others were associated with cheese lacking in flavor. In general, cheese containing comparatively large amounts of volatile acid had more of the sharp, peppery flavor characteristic of blue cheese than cheese containing small amounts. There was a definite difference in quality of the cheese produced by various strains identified as *P. roqueforti.*

It is probable that various mold species can bring about the general changes necessary in the ripening of blue cheese, although *P. roqueforti* has been most useful for this purpose.

Factors influencing the growth of *P. roqueforti*. The great variations in the mold growth in the blue-veined cheeses suggest there are various influencing factors. Golding[148] noted that citric and acetic acids had an effect on the growth of different strains of *P. roqueforti*. Low concentrations of acetic acid tended to reduce digestion of casein in milk, while citric acid tended to increase it. In a medium made up of distilled water, various salts, peptone, lactose, and agar, acetic acid increased the growth, whereas citric acid tended to inhibit it. Studies by Golding[149] on salt tolerance of *P. roqueforti* suggest that the salt concentrations usually found in blue cheese may retard but not inhibit growth of the mold. Composition of the cheese may have such an influence on the growth of *P. roqueforti* that it is an important factor in determining development of the mold under practical conditions. Golding[150] subjected blue cheese to high pressures in an iron cylinder during ripening, using both continuous and intermittent treatment. The procedures accelerated the mold growth somewhat but the intermittent method gave the better results.

More recently, Golding[151] concluded that it is presence of carbon dioxide rather than absence of oxygen that is a limiting factor in the growth of *P. roqueforti* in cheese, and that punching the cheese aids the mold growth by permitting escape of carbon dioxide rather than entrance of oxygen. Three strains[152] of *P. roqueforti* were grown at seven temperatures in atmospheres from 21 to 2.1 per cent oxygen, obtained by adding nitrogen to air. It was only with the greatest dilution of oxygen (2.1%) that a significant reduction in growth was noted; it ranged from 16 to 54 per cent. It appeared there is a tendency for a shortage of oxygen to reduce growth more at higher temperatures. Relatively small concentrations of carbon dioxide in air increased the growth of *P. roqueforti* and larger concentrations inhibited it.[153] Acceleration of growth due to low concentrations of carbon dioxide and inhibition due to high concentrations were apparent sooner at lower temperatures.

Factors influencing ripening. By homogenizing cows' milk from which blue cheese was made, Lane and Hammer[154] greatly accelerated the rate of ripening. Finely divided fat evidently hydrolyzes more readily than fat in the usual condition so that the changes in the fat progress more rapidly. Homogenization also results in a soft body, which permits spreading of the cheese, and in comparatively white color. When homogenized milk is used, fat hydrolysis due to action of milk lipase often is evident before the curd is in the hoops and may be a factor in accelerating the ripening. Much of the blue cheese now made in the United States is made with homogenization of the milk or with a mixture of skim milk and homogenized cream.

Coulter *et al.*[155] noted that, within the range of the values used, variations in acidity during manufacture of blue cheese did not appear to be highly important. Cheese set and dipped at low acidities ripened somewhat sooner than those set and dipped at high acidities but excellent cheese was produced with all the acidities used.

According to Coulter and Combs,[156] addition of steapsin to the milk or the curd accelerated the ripening of blue cheese. With commercial steapsin added, approximately the same flavor development was obtained in 5 mo as the control cheese showed in 12 mo. Steapsin gave the cheese a bitter flavor. Cheese made with steapsin but without mold became rancid but did not develop the typical blue cheese flavor, so that while steapsin accelerates ripening the typical flavor requires action of the characteristic mold.

Lane and Hammer [157] found that addition of 2 per cent salt to blue cheese curd just before hooping sometimes resulted in better mold growth in the cheese, rather regularly gave a lighter color, and tended to control certain flavor defects. The better mold growth probably was due to the more open texture resulting from the salting, the lighter color to an increase in stability of the curd that kept the fat dispersed, and the tendency to control flavor defects to action of salt on certain undesirable organisms. Some of the salt added to the curd drains away in the whey and, although the percentage lost is not constant, no serious irregularities occurred in the salt content of the finished cheese.

Thiobodeau and Macy [138] reported that addition to blue cheese at hooping of the enzymes of *P. roqueforti* in the form of mycelium (6 gm per 5-lb cheese) produced cheese of fine quality in one-half the normal ripening period.

Defects of blue cheese. Because *P. roqueforti* is the primary ripening agent in blue cheese, its proper development is very important. If the mold does not develop sufficiently, the cheese lacks color and flavor and is too firm. In some cases the mold in an uncut cheese loses its color but regains it relatively quickly when exposed to the air. Excessive mold growth in blue cheese commonly results in a musty, unclean flavor and the cheese lacks the characteristic flavor of methyl-*n*-amyl ketone.

Proper mold development in blue cheese is dependent on proper texture in the cheese and adequate control of the ripening conditions. The strain of mold used also is of extreme importance. An outbreak of lack of mold growth apparently was due to use of an atypical strain of *P. roqueforti* in preparing the mold powder.[158]

Gas holes occasionally are noted in commercial blue cheese but the defect is less common than might be expected since blue cheese so often is made from raw milk. The open texture of blue cheese may be a factor. Bryant and Hammer[158] reported that trials with a culture of *A. aerogenes* recently isolated from gassy cheddar cheese showed that inoculations into milk which resulted in very gassy cheddar cheese caused no significant gas holes in blue cheese; the blue cheese was made from homogenized milk and early production of fatty acids may have had an inhibitory effect on the gas-forming organisms.

FIG. 47. Blue cheese curing cave. Courtesy F. M. Frederiksen.

Blue cheese occasionally develops a defect in which a portion or all of the edges of a cheese become soft.[158] There is no gradual softening as the defective edge is approached and the dividing line between firm and soft cheese is sharply defined. The surface of the soft edge is readily slipped off, exposing soft cheese beneath that has the color and consistency of camembert; with age, the odor of the soft cheese suggests limburger. The soft edge has a higher moisture and lower salt content than the normal cheese and also a higher *p*H and higher bacterial content. The defect has been produced by placing normal cheese near a humidifier where free moisture could strike it. Various conditions in curing rooms, such as occasional excessive cooling of cheese near brine coils, might result in accumulation of moisture on cheese. Softening would be expected to occur most readily at an edge since the two surfaces are separated by a relatively small amount of cheese and the ratio of deposited moisture to cheese is high.

In blue cheese that has ripened for some time, gray discoloration is a rather common defect.[158] It is most commonly noted at the surface, especially when the slime is removed, and tends to spread through the entire cheese. Extensive discoloration usually is accompanied by a mousy, ammoniacal flavor that becomes soapy with age. The defect seems to be confined to certain lots of cheese but may not involve all the cheese in a lot. It does not appear to spread from one cheese to another. The defect appears to be accompanied by an increase in *p*H. The variation from the normal ripening mechanism that causes the defect presumably involves formation of basic products from protein; extensive development of *P. roqueforti,* or growth of contaminating organisms, could be responsible for an abnormal protein decomposition. Contaminants capable of reproducing the defect have not been isolated.

A black discoloration and musty flavor in blue cheese have been attributed to growth of *Hormodendrum olivaceum,* particularly in punch holes and surface cracks.[158] Under experimental conditions growth of the mold was confined to the outside of the cheese and to punch holes but it appeared that with cheese having a bodv susceptible to cracking the mold could cause extensive damage.

In certain curing rooms red areas of mold growth often appear on the cheese. Commonly, the mold is first evident as small bright-red colonies that may be few or very numerous. The colonies usually are evident in 10 days to several weeks and increase in size until they are 1 in. or more in diameter. They may grow together and form large red areas which occasionally make up a considerable portion of the cheese surface. Hammer and Gilman [159] easily isolated the mold on a special cheese agar but not on various other media. They considered the designation should be *Sporendonema casei,* although various other names have been employed. Covering the cheese in various ways, for example, with wax or with petrolatum containing mold inhibitors, aids in preventing growth of the mold when the normal cleaning of the cheese does not.

LIMBURGER CHEESE

Limburger cheese is a soft, bacteria-ripened variety that was first made in Belgium. It is characterized by a surface smear that plays an important role in the ripening.

Lactic acid streptococci. There is a rapid drop in *p*H of limburger cheese after manufacture because of the activity of the lactic acid streptococci present in the milk used or added as culture; this activity is favored by the relatively high moisture content of the cheese and by the holding temperature. Yale [160] found the *p*H drop was most rapid during the 24 hr following dipping. At the end of that period the *p*H values of cheese made from raw milk without culture ranged from 4.72 to 5.04 in July and August and from 5.08 to 5.10 in October. At 7 days, values for the July and August cheese were slightly higher than at 24 hr and, with one exception when there was a drop in *p*H, they ranged from 5.05 to 5.23. As the cheese aged, the *p*H values increased and ranged from 6.53 to 6.98 at 70 days. When the cheese were fully ripe (70 days), average *p*H values of July and August cheese and of October cheese were about the same, being 6.79 and 6.64, respectively. Undoubtedly, the cellar temperatures are a factor in the *p*H changes. Pasteurization retarded but did not stop acid development. At 24 hr, cheese made without culture from (1) raw milk, (2) milk heated to 62.8 C (145 F) without holding, (3) milk held 15 min at 62.8 C, and (4) milk held 30 min at 62.8 C had *p*H values of 4.72, 4.85, 4.95, and 5.05, respectively. At 7 days, the values were about the same for raw- and pasteurized-milk cheese. Addition of 0.05 to 0.2 per cent culture to partly or completely pasteurized milk hastened acid development so that at 24 hr the *p*H values usually were about 5.0, being appreciably lower than without culture. During ripening, *p*H values of cheese from milk containing culture tended to increase more slowly than the values of raw- or pasteurized-milk cheese made without culture. In one series, *p*H values at 70 days were 6.90 and 6.95 for cheese made without culture, compared to 6.16 and 5.89 for cheese made with culture.

Kelly and Marquardt [144] noted that the *p*H was not uniform throughout a cheese. In general, the *p*H was higher just beneath the surface than at the center. As soon as a definite smear developed on the cheese, the *p*H of the smear was higher than that of the cheese itself, due to activity of the organisms there.

Smear of cheese. Development of a normal smear is an important factor in the ripening of limburger cheese. Kelly [161] found that the microbiological changes on the surface of ripening cheese showed a very definite sequence. Budding yeasts appeared in 2 to 3 days and were in large masses in 4 to 5 days. At this stage the cheese surface was slimy and the organisms could be distributed evenly over it by rubbing. In 6 or 7 days, *Bact. linens* appeared and by about the eighth day it had increased greatly and was evenly distributed over the whole surface; apparently, this organism was responsible for the reddish surface color that developed at this period. In 10 to 18 days, the yeast cells tended to become smaller and distorted and then disappeared entirely. Very few yeast cells were found on the older cheese. Other organisms sometimes were present in the smear but did not appear to be important in the ripening. The predominant yeast formed a pellicle on liquid media, tolerated salt in concentrations as high as 18 to 20 per cent, and did not produce spores.

Yale [162] determined at two factories the numbers of organisms in the smears of limburger cheese from 2 to 14 days old. The maximum yeast counts of 900,000,000 and 6,300,000,000 per gram were found on the fourth day; both counts were followed by decreases. The maximum bacterial counts of 62,000,000,000 and 360,000,000,000 per gram were found on the tenth and fourteenth days, which were the last examinations. Many of the bacteria in the smear were salt-tolerant and grew on a medium containing 7.5 per cent salt. The predominant group of bacteria in the smear was strictly aerobic, Gram-positive, non-chromogenic, non-spore-forming rods that produced a marked alkaline reaction in litmus milk in 1 mo at 25 C (77 F) and did not liquefy gelatin. *Bact. linens* was next in importance. Inoculation of the surface of raw-milk limburger cheese with *Bact. linens* failed to improve the quality of the cheese over that naturally inoculated under good plant conditions, but addition of milk cultures to pasteurized milk improved the flavor in the majority of the lots of cheese made in four trials at two factories.

The conditions that largely determine the flora of the smear include salt concentration and *p*H. Kelly and Marquardt [144] outlined the sequence of changes involved. Soon after salting, the salt concentration is highest in the surface layer of the cheese and low in the interior; the gradual penetration results in an equilibrium in about 10 days. Organisms on the surface of young cheese are in a high concentration of salt and this excludes many types while the low *p*H excludes still others. The yeasts on the cheese tolerate both conditions and act on lactate and protein to raise the *p*H above the point (*p*H 5.85) at which *Bact. linens* can grow. The high salt concentration still prevents growth of many species. *Bact. linens* raises the *p*H still higher through its action on proteins and is believed to complete the ripening. The aerobic nature of *Bact. linens* and its inability to grow below *p*H 5.85 presumably prevent its development in the interior of the cheese; this suggests that the buttery texture and some of the flavor are produced by enzymes of *Bact. linens* rather than by the organism itself.

Apparently, under the usual conditions the cheese are inoculated with the needed surface organisms from equipment, shelving, etc., in the plant. In addition, the organisms probably are present, at least in small numbers, in the milk coming from the farms.

Pasteurization of milk. Yale[160] found that either partial or complete pasteurization markedly improved the quality of cheese made in July and August from poor-quality milk but not that made in October from better-quality milk. Heating 30 min at 62.8 C (145 F) resulted in as good cheese as partial pasteurization, using holding periods of 0, 10, or 20 min. Cheese from pasteurized milk had a milder limburger flavor than that from raw milk. Flavor development in the former varied with the different lots and usually was most pronounced without added culture. With some lots, culture was not beneficial, whereas with others addition of 0.05 to 0.1 per cent improved the grade of the cheese. Pasteurization of milk with proper manufacturing methods resulted in a product of uniform quality.

Defects. Investigations of Kelly[163] showed that gas in limburger cheese was due to growth of *E. coli* and *A. aerogenes*. The relatively high temperatures maintained during the making and the low acidity of the milk encourage growth of these organisms so that gas was produced in the cheese when as few as 12 per milliliter were present in the milk. More gas was produced when the proportion of coliform organisms to other organisms was high than when it was low. Good cheese was made from milk heated sufficiently to control gas-producing organisms.

Kelly[161] occasionally encountered *Oospora* on limburger cheese, and whenever the numbers were significant the cheese showed the wrinkled appearance that usually occurs with extensive development of *O. lactis*.

Kelly and Marquardt[144] studied two lots of cheese in which the drop in *p*H did not go below 6.0 and was followed by a steady rise; the cheese developed a strong putrid odor. The results indicate acid development is necessary to control putrefactive bacteria.

In the experiments of Yale,[160] use of large amounts of culture in heated milk gave cheese which developed a body, texture, and flavor more like brick than limburger cheese.

Gas production by cheese in tins. Dorn and Dahlberg[50] found that the volume of gas produced by limburger cheese in tins was greatly increased by allowing the cheese to ripen for 2 weeks under aerobic conditions before canning. This preliminary ripening gave cheese of good quality, including a smooth body. With canning immediately after salting, the curd remained acid and never ripened. Vacuum packing, compared with sealing at atmospheric pressure, had but slight effect on rate of gas production or types of gases produced; the total volume of evolved gas tended to be slightly lower with vacuum packing. With fresh limburger curd, carbon dioxide constituted up to 95 to 97 per cent of the gases given off and hydrogen 3 to 4 per cent. With partially ripened curd, the volume of gases was 4 to 5 times as great as with fresh curd; carbon dioxide made up 90 to 95 per cent and hydrogen 5 to 10 per cent. The gases evolved per pound of cheese canned when partially ripe were 2 to 3 l during 18 weeks at 10 C (50 F) and 1.5 l at 4.4 C (40 F); they represented a loss of 0.6 to 1.2 per cent of the weight of the cheese. The steam distillate of the cheese contained ammonia and indol. Hydrogen sulfide was present in the gases evolved. The volatile fatty acids of the cheese included acetic and propionic but were chiefly higher fatty acids, probably resulting from fat hydrolysis.

CAMEMBERT CHEESE

Camembert cheese is a soft, mold-ripened type that originated in France. Molds, and commonly bacteria and yeasts, grow at the surface of the cheese and bring about the ripening through elaboration of enzymes that diffuse toward the center of the cheese. Since the ripening follows these enzymes rather closely, the outer portion of the cheese ripens first; often there is a sharp differentiation between the creamy, waxy, ripened cheese and the unripened curd.

Lactic acid streptococci. The lactic acid streptococci are primarily responsible for formation of acid in the cheese. Their abundance in the fresh curd is assured through use of culture in the milk. They encounter conditions very favorable for growth because of the retention of considerable quantities of water and materials in solution, and continue to develop until large numbers are present. Esten and Mason [164] found that the highest counts usually occurred about the third day, when there were up to several billion organisms per gram. A decrease then began and continued, with fluctuations, until the cheese was ripe. Usually 99 per cent or more of the bacteria in the interior of the cheese was of the lactic acid type.

Organisms other than *S. lactis* in the cheese mass. Various microorganisms other than *S. lactis* are present more or less constantly in the cheese mass. Esten and Mason [164] found liquefying bacteria, sometimes in considerable numbers, lactobacilli, and yeasts. In general, these organisms are present because of their ability to develop in an acid medium; they apparently play no part in the ripening.

Molds of camembert cheese. *Penicillium camemberti* generally is regarded as the characteristic mold of camembert cheese; a white variety has been designated *P. camemberti* var. *rogeri.* The species seems to be largely limited in its growth to camembert cheese and is not widely distributed.

Early studies on camembert cheese suggested that, for normal ripening, growth of both *P. camemberti* and *O. lactis* is required on the surface. According to this idea, if *O. lactis* is absent or limited in its growth, ripening may appear to progress normally but the cheese lacks flavor, whereas if overdevelopment occurs surface growth on the cheese is thick and wrinkled, it does not adhere properly, and *P. camemberti* fails to develop as it should. Nutrients and moisture available at the surface of the cheese are very satisfactory for *O. lactis* but the salt present tends to restrain it because of its sensitivity to this compound.

A later idea is that growth of *O. lactis* is not necessary in the ripening of camembert cheese and the product has been made with extreme precautions to limit growth greatly on the cheese surface of all organisms except *P. camemberti;* these precautions include pasteurization of the milk, thorough steaming of all the equipment with which the milk or cheese comes in contact, heavy inoculation of the young cheese with *P. camemberti,* and great care in the curing rooms. Some of the cheese made on this general basis has been satisfactory for many consumers but various defects have been encountered in certain lots.

Another recent idea is that *O. lactis* is the important organism and *P. camemberti* is not necessary. On this basis precautions are taken to avoid contamination with other molds, and *O. lactis* grows very rapidly at the favorable temperature and high humidity provided. Some of the cheese made in this way are very acceptable to many consumers, while others do not regard it as typical camembert.

If *P. camemberti* is to be inoculated on the fresh cheese, it can be grown on water crackers that have been sterilized in a container and then moistened with sterile water to which mold from a stock culture has been added; in some instances a 5 or 10 per cent aqueous solution of lactic or tartaric acid is used instead of the water. The cheese can be inoculated by sprinkling with, or spraying on, a water suspension of the mold. Some makers believe it is unnecessary to inoculate after the mold is once established in a plant because the organism is added to the cheese from the air and equipment.

P. camemberti soon is evident on the cheese and then spreads rapidly over the surface. Later, when spore formation begins, the mold may be still more conspicuous.

Other surface organisms. Along with the growth of molds at the surface of camembert cheese there commonly is growth of yeasts and bacteria. *Bact. linens* appears to be of special importance in this connection and the reddish color often noted on the surface of camembert cheese probably is most commonly the result of growth of this organism. Eventually, growth of the various organisms results in formation of a slime in which the molds are not especially conspicuous.

Esten and Mason [164] determined the numbers of bacteria and yeasts in the slime of camembert cheese, both imported and domestic, and found approximately 7 to 259 billion bacteria per gram and 650,000 to 36,000,000 yeasts. In all probability these organisms do not play a necessary part in the ripening process; however, they tend to limit mold growth and may form a coating that partially excludes the air and thus aids in controlling off flavors.

Conditions provided at the surface of camembert cheese during ripening are very favorable for the development of many species of micro-organisms. Humidity of the ripening room normally is high, nutrients are available from the cheese mass, and the lactic acid supplied through activity of *S. lactis* favors certain yeasts and molds. It is probable also that associative action among the various species makes possible an extensive development of certain of them.

Importance of molds. In distinction to the blue-veined cheeses, the necessary mold growth in the camembert type is limited to the surface. The principal action of the surface mold is to bring about proteolysis and flavor development. The proteolyzing action of *P. camemberti* can be shown by inoculating sterile curd; the decomposition products formed are essentially the same as those in camembert cheese. The enzymes produced diffuse from the surface growth and, through their action on casein and casein derivatives, bring about pronounced changes that influence the texture and flavor. At certain stages the outer portion of the cheese is ripe while the inner portion still is green. *P. camemberti* also is active in reducing the acidity in the cheese; this occurs through use of acid for the energy that can be obtained and through neutralization by the compounds formed.

Proteolysis in camembert cheese. The principal proteolytic agents in camembert cheese are the enzymes produced by the surface mold. Bosworth [165] pointed out that the only function of the rennet is to coagulate the milk. He found that the water-soluble nitrogen does not increase appreciably until the cheese is 10 days old, but that finally the proteolysis is extensive, more than 80 per cent of the nitrogen being water soluble after 4 weeks. A considerable amount of amid nitrogen is formed and, toward the end of the ripening, some ammonia. The odor of ammonia sometimes is very evident in ripened camembert cheese.

Numbers of bacteria in ripened cheese. Although the maximum numbers of bacteria in camembert cheese are present soon after making and a decrease then begins, there may be large numbers in the cheese mass when ripening is complete. Esten and Mason[164] found that four samples of ripened, domestic camembert cheese had approximately 45 million to 2.3 billion bacteria per gram, and four samples of imported cheese had approximately 6 to 442 million. The variation in these counts is large. The decrease in numbers from the maximum is due to development of unfavorable conditions for the bacteria, and variations would be expected from one cheese to another; undoubtedly, age of the cheese also is a factor.

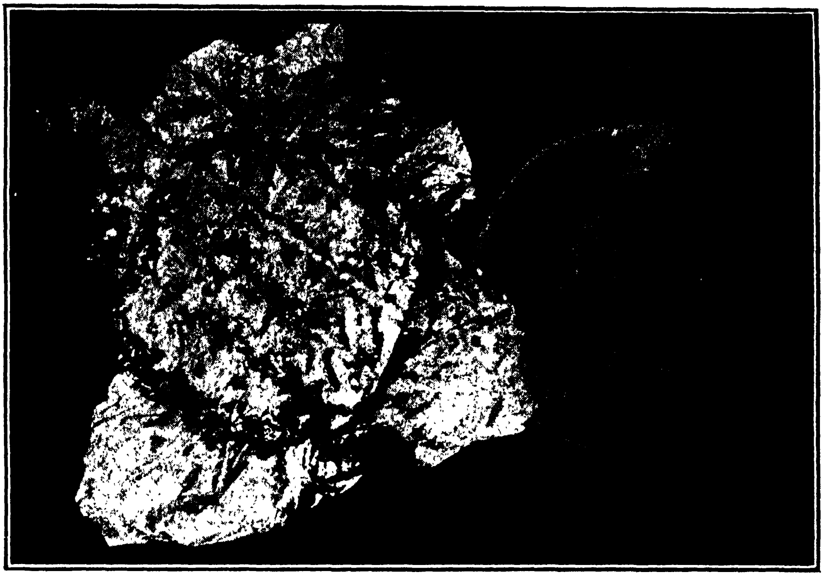

FIG. 48. Open package of camembert cheese (domestic) showing normal surface growth.

Manufacture of cheese from pasteurized milk. Hochstresser and Price[166] reported that milk pasteurized at 62.8 C (145 F) for 30 min produced camembert cheese of a better average quality and greater uniformity than raw milk. Improvement was greatest when the original milk was of inferior grade. The quality of the pasteurized-milk cheese improved as the quality of the original milk improved. The investigators noted that pasteurization had practically no influence on the yield of ripened cheese.

Defects. Camembert cheese may fail to develop a creamy, waxy texture and remain hard, often with darkening of the cheese mass; presumably, this is due to failure of the cheese to develop the proper proteolytic enzymes in the surface growth. Irregularities in appearance of the surface growth include areas of objectionable color attributable to contaminating molds and other organisms; dark molds [167] and pink yeasts are especially conspicuous. The young cheese may not retain their normal shape because of insufficient acid development. Camembert cheese that is held too long may develop objectionable flavors, due to overripening by the normal organisms or to contamination.

COTTAGE CHEESE

Cottage cheese belongs to the group of soft, unripened cheeses in which curdling of the milk is due primarily to acid development, although with most methods of manufacture a small amount of coagulating enzyme added to the milk at setting is a contributing factor. The usual commercial practice is to pasteurize the milk, and then add lactic acid culture and a small amount of rennet or special coagulator. Often culture that is especially active in acid production is employed. The amount of culture added is dependent not only on its activity but on whether a short or long set is to be used; with a short set—3 to 5 hr ripening at 29.4 to 33.3 C (85 to 92 F)—2 to 5 per cent or more culture may be needed, whereas with a long set—12 to 16 hr ripening at 21.1 to 26.7 C (70 to 80 F)—much smaller amounts are used. When the milk is coagulated and the whey acidity satisfactory, the curd is cut and cooked and the whey drained. Washing the curd removes some of the retained whey and also greatly lowers the temperature so that it is in better condition for holding; the final wash water should be well chilled. Since the changes yielding the typical flavor largely occur during coagulation of the milk, the cheese is ready to eat as soon as it is made. Commonly, cream and salt are added to the curd and the product sold as creamed cottage cheese.

Sources of flavor. The typical flavor of cottage cheese is due to the milk constituents present and to products of growth of the culture bacteria during coagulation of the milk, especially acid. Extensive washing of the curd removes a portion of these products and lactic acid apparently is the one product of primary importance in the fresh cheese. Because of this, pure cultures of lactic acid bacteria may yield cheese that is satisfactory for certain markets. On the other hand, some consumers prefer a high flavor and small amounts of biacetyl formed during coagulation of the milk definitely contribute to this flavor. Biacetyl also can be formed during holding of the cheese and in some instances its odor is very conspicuous. Small amounts of added citric acid tend to increase the biacetyl content, just as with lactic acid cultures; unless regulations prevent they can be added when the cheese is creamed.

Pasteurization of the milk. In the manufacture of cottage cheese, efficient pasteurization of the milk is important. Without it, the flavor, and also the body and texture, of the product may be greatly influenced by various organisms in the milk that find opportunity for growth during the period the milk is being held at a temperature favorable for acid development by the culture organisms inoculated. Gas-forming organisms, especially those of the *Escherichia-Aerobacter* group, may be so active that the curd is filled with gas holes. Hammer[168] reported gas formation in a vat of milk set for cottage cheese that was so pronounced the gassy curd rose over the edge of the vat. Gas-forming organisms ordinarily produce a very undesirable flavor. Recontamination of heated milk from equipment, etc., may be as serious as inefficient pasteurization; also, objectionable organisms may be added to the curd from contaminated utensils, wash water, hands and clothing of operators, air, etc.

Either LTLT or HTST pasteurization can be used with milk intended for cottage cheese; the latter definitely simplifies an operation in which a number of vats are set.

Importance of the culture. The culture used is of extreme importance in cottage cheese manufacture since acid development must be reasonably rapid for the most satisfactory results. Slow cultures, due to presence of phage or other causes, lower the quality of the cheese and upset manufacturing schedules; also, they may cause losses of vats of cheese because with poor acid development the cheese is unsatisfactory. Undoubtedly, inadequate cultures are the greatest hazard in cottage cheese manufacture.

Cultures for use in cottage cheese production can be tested for activity with a simple procedure that shows the rate of acid development.

Details of the test, such as temperature and per cent culture employed, are determined by the exact manufacturing procedure followed. If 30 C (86 F) and 5 per cent culture are being used for the cheese, an activity test on a culture can be carried out as follows. A small container that can be stoppered is sterilized and partly filled with a measured amount (for example, 180 ml) of pasteurized milk which is ready for the cheese vats. The temperature is adjusted to 30 C in a water bath and 5 per cent culture (9 ml for 180 ml milk) added with a sterile pipette and distributed through the milk. The mixture is titrated at once and after 2 and 4 hr, the temperature having been maintained at 30 C. Perhaps the most significant value is the acidity after 4 hr, although the increase in acid from 2 to 4 hr is very important. The culture used for cheese should be active enough to coagulate the milk and have it ready for cutting in the desired period.

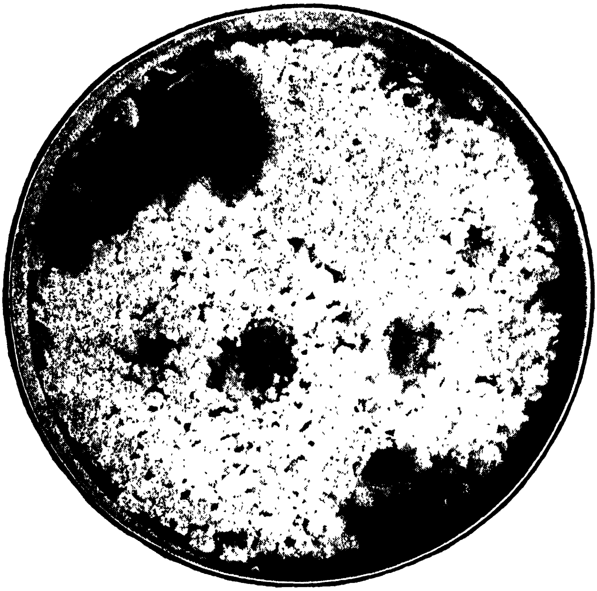

FIG. 49. Mold growth on cottage cheese.

Deterioration. The acid present in cottage cheese inhibits development of many species of micro-organisms, but others, especially molds and yeasts, tolerate the acid and their growth may even be accelerated by it; the high percentage of water in the product also tends to favor their activity. Accordingly, cottage cheese has poor keeping qualities at the temperatures encountered in retail establishments and homes.

Molds sometimes appear at the surface of the cheese and in air spaces along the wall of the container. There may be many centers of growth, if unsatisfactory methods have been used, or only an occasional one suggestive of air contamination. The color and general type of growth vary greatly because of the variety of species involved. *Oospora lactis* and *Penicillium* species appear to be the most common. Molds not only spoil the appearance of the cheese but also influence the flavor; certain of them have pronounced proteolyzing powers and, if given time, digest considerable portions of the curd.

Some yeasts develop only where there is an abundant air supply; others are capable of growth through the cheese mass. Considerable surface growth of the species forming white colonies can occur without being especially noticeable, because of lack of contrast. Colonies of pink yeasts are very conspicuous; in general, these organisms have little effect on the flavor of the cheese, even when growth is abundant. A typical yeasty flavor usually is due to the lactose-fermenting species.

In a survey of open market and plant samples of cottage cheese, Foter *et al.*[169] found that the most common spoilage organisms were yeasts which varied in number from 0 to 24,000,000 per gram. Of the total samples, 31 per cent were yeasty, 22 per cent sour, and 20 per cent bitter; about 10 per cent were not criticized for flavor.

Bacteria cannot be excluded as a cause of deterioration in cottage cheese and in some instances bacteria other than culture types are present in enormous numbers. Their growth may be favored by reduction in the acid at certain points through activity of molds and yeasts. *Ps. fragi* has been found the cause of a fruity odor in cottage cheese; it was much more numerous at the surface of the cheese than in the interior.

Preservation. The principal method of preserving cottage cheese is through use of low temperatures. It can be held at 0 C (32 F) for a considerable period without showing conspicuous changes, but at 10 C (50 F), or higher, it keeps for a much shorter period. Controlled methods of making and packing aid in preventing entrance of undesirable organisms and thus have an effect on the keeping quality.

Cottage cheese curd keeps better than creamed cheese. Organisms added with the cream may be a factor. Jensen [170] placed fresh cottage cheese curd in brine concentrations from 0.0 to 5.0 per cent and stored it at 7.2 C (45 F). In 3 to 4 per cent brines the curd retained its fresh flavor for 14 days, whereas lower concentrations resulted in flavor deterioration. Curd in fresh water rapidly lost its flavor. Curd held dry deteriorated the most rapidly. The texture of curd stored in 3 to 4 per cent brines was softer and more pliable than that of curd held in brines of lower concentrations. Fouts *et al.*[171] stored cottage cheese curd at 1.7 to 7.2 C (35 to 45 F) in 2, 4, and 6 per cent brines. Four per cent brine preserved the curd satisfactorily at 1.7 C for 2 mo and it was salable as creamed cottage cheese considerably longer. Regardless of the brine concentration, the curd at 7.2 C was satisfactory for only 1 mo; after 2 mo mold was present and the curd had a fermented flavor. Storing curd in brine requires a container which will not corrode readily. Olson[172] reported cottage cheese curd can be stored up to 2 weeks at 4.4 C (40 F) in 3 per cent brine with little deterioration.

Cottage cheese sometimes is packed in large containers and frozen so that it can be held for considerable periods. When thawed, the curd is mixed with cream. Commonly the freezing and thawing break up the curd. The cheese often keeps very well, but off flavors, such as high-acid, stale, bitter, metallic, etc., may develop, owing to growth of organisms before the cheese is frozen, or to chemical action. Ellenberger[173] noted that the keeping quality of cottage cheese after removal from storage was nearly, although not quite, as good as that of fresh cheese.

Marquardt[174] reported that cottage cheese curd for freezing should be prepared from pasteurized milk and should be thoroughly washed and lightly salted (25 to 50% of the salt that is desired in the finished product) but never creamed. After it is firmly packed in 50-lb tins, immediate freezing at −17.8 to −28.9 C (0 to −20 F) is important. The tins should be stacked open for 48 hr, with a fan operating in the room, after which they can be stacked in solid rows. In thawing, the tins should be placed in water at room temperature and the water agitated. Complete thawing requires 2 to 4 days. The thawed curd is mixed with salt and cream. Fresh curd may be mixed with the thawed material. Off flavors in the finished product may be due to high salt and acid, enzymes, or bacteria.

Laboratory examination. In order to control the microbiological condition of cottage cheese, it can be examined by the plate method for (1) yeasts and molds, and/or (2) bacteria other than lactic acid culture types. Ordinarily, all the latter organisms cannot be accurately differentiated from the culture organisms by the colony appearance but if relatively large numbers are present they are easily detected. Fresh cheese commonly contains only small numbers of contaminating organisms because the combination of the cooking temperature and the acid in the curd destroys most of those present when the cooking is begun, even when the numbers are high. However, the product is readily contaminated during handling from such sources as improperly pasteurized cream, hands and clothing of employees, contaminated equipment, etc., and, unless kept cold, growth of the contaminants begins.

Cottage cheese that shows flavor deterioration should be plated when it appears organisms are involved. From the appearance of the colonies that develop and/or from microscopic examination of them, the general types of organisms responsible can be determined. Direct microscopic examination of the defective cheese often is helpful in indicating the type of contamination causing a flavor defect.

VITAMINS IN CHEESES

In connection with studies on the vitamin contents of various food products, many samples of cheeses have been examined. Some of the earlier results may be open to question, because of the methods used, but the more recent ones have been obtained with adequate procedures. Wide variations occur in the vitamin contents of different cheeses. Hathaway and Davis [175] noted considerable variation in vitamin A contents of 22 kinds of cheeses, and also [176] in riboflavin contents of 27 kinds of cheeses and cheese spreads.

During their manufacture cheeses retain a portion of the vitamins in the original milk, with the remainder, primarily the water-soluble vitamins, being left in the whey. The vitamin contents of certain cheeses are increased through activity of the organisms normally present. Such increases involve soft and semi-hard types, rather than hard types. Production of vitamins by the organisms developing in or on cheeses offers some interesting possibilities in connection with cheese ripening and, eventually, may be considered in developing curing procedures.

Retention of vitamins. Higuchi *et al.*[177] noted that in cheddar cheesemaking 85 per cent of the vitamin A potency was retained in the cheese, 7 per cent was in the whey, and 8 per cent was not accounted for; the results apply to both carotene and vitamin A. The vitamin A potency of cheese during curing and storage was practically unchanged over almost 1 yr. Cheese made from milk produced on an alfalfa silage ration equaled or excelled in quality and vitamin A potency cheese made from milk produced on a corn silage ration.

Guerrant *et al.*[178] noted a measurable decrease in vitamin A potency during ripening of cheddar cheese but the loss was almost wholly compensated for by moisture losses.

Irvine *et al.*[179] found that 23 per cent of the riboflavin originally in the milk was retained in Canadian cheddar cheese made from raw milk.

Riboflavin appeared to be stable throughout 12 mo ripening, both at 4.4 and 14.4 C (40 and 58 F). Pasteurization[180] by either the LTLT or the HTST method had no significant effect upon retention of riboflavin, when compared to raw-milk cheese; values after 8 mo ripening were about equal to those in the fresh cheese. With brick cheese[181] 27.4 per cent of the riboflavin originally in the milk was retained in the cheese; with blue cheese the value was 30.1. Riboflavin appeared to be quite stable during the ripening of brick and blue cheeses.

Production of vitamins. Sullivan *et al.*[182] analyzed 12 different types of cheeses for riboflavin, pantothenic acid, nicotinic acid, and biotin. Only minor variations were noted in the concentrations of riboflavin, but the other factors appeared to be quite dependent upon the age of the cheese and the ripening process involved. Pantothenic acid, nicotinic acid, and biotin values were especially high with camembert and blue cheeses. During ripening of limburger cheese, there was little change in the riboflavin values but a definite increase in pantothenic acid, nicotinic acid, and biotin.

Burkholder *et al.*[183] reported that organisms isolated from camembert, liederkranz, brie, and limburger cheeses synthesized considerable quantities of B vitamins in chemically defined media. Thiamin, riboflavin, biotin, and nicotinic acid appeared to increase on the surfaces of these cheeses through progressive stages of ripening.

Todhunter *et al.*[184] determined the vitamin A content of roquefort-type cheese, with and without mold development, and found no evidence that mold growth increased the vitamin A value.

SLOW ACID PRODUCTION IN CHEESEMAKING

In the making of various cheeses, slow acid production is a more or less constant hazard. Certain plants frequently encounter this irregularity; others escape it for long periods and then suddenly have difficulty. Slow acid production varies widely in degree. In some cases acid formation is greatly delayed, whereas in others it is only slightly delayed. With the extreme condition the vat of milk is lost or is made into very poor cheese; with only a slight delay the most important consideration is the longer period required for completion of the cheesemaking operation.

The common cause of slow acid production is bacteriophage (Chapter 14). In outbreaks of phage, use of cultures from plants or laboratories that did not supply the one involved in the outbreak is an accepted procedure that repeatedly has restored normal plant operations.

The new cultures are resistant to the strain or strains of phage that have become established in the plant and develop acid actively. Certain plants find it advisable to keep in reserve, in a frozen condition or otherwise, a culture from a new source for use when the regular culture fails.

Phage is retained in cheese and its persistence there may be a factor of importance in outbreaks of slow acid production in cheese plants. Nichols and Wolf[185] found that several cheeses made after the culture had failed or during outbreaks of slow acid production at three different plants contained phage; the concentration of phage was fairly constant when tested periodically during 1 yr. They noted that since phage persists for such long periods in cheese it can be carried over from one season to the next.

Milk from infected udders and organisms that have an inhibitory effect on the culture bacteria are other possible causes of slow acid production. Apparently, these two causes are only occasionally involved and then are responsible for only small delays in acid formation. Because of the peculiar effects sometimes produced by phage, it appears that difficulties due to phage may be ascribed to other causes, such as quality of the milk.

Variations in heat resistance of lactic acid organisms and particularly the influence of various factors on the resistance may result in an unusual effect of the cooking temperature on the activity of the culture organisms. Harrison and Dearden[186] suggested that inability of certain strains of streptococci to grow at cooking temperatures may be one cause of slowness in cheesemaking. Hunter[187] noted that with some strains of lactic acid organisms variations were well marked, including response to high temperature conditions.

Activity tests can be run on lactic acid cultures intended for one or more of the ripened cheeses. They differ from the activity tests on cultures for cottage cheese since ability of the culture organisms to survive the cooking temperature and retain their acid-producing power is essential. The cheesemaking operation[188] can be simulated with a small amount of milk in a sterile milk bottle or other container, using all the details of the manufacturing procedure. Conclusions are based on whey acidities or pH values at various stages in the operation and/or *p*H values on the curd at various ages. The tests permit comparisons of a number of lactic acid cultures at any one time and also make it possible to check the activity of the culture used in the operations on different days.

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Chapter 18

Bacteriology of Dairy-Plant Water Supplies

The bacteriological quality of the water that comes in contact with dairy products, or with equipment in dairy plants, is important for two distinct reasons: (1) The water may contain pathogens that are left in the dairy products and/or (2) the water may contain organisms that cause spoilage in the dairy products. An additional reason for controlling the bacteriological quality of dairy-plant water supplies is the health of the employees. Dairy-plant employees often drink large volumes of water, particularly when working at comparatively high temperatures.

Organisms in water supplies get into a dairy product most readily by direct contact with the product. The organisms from such sources that are left on dairy-plant equipment normally are killed when it is sterilized. However, in some instances attempted sterilization fails, through carelessness or the difficulties involved, and the surviving organisms then get into the dairy product when the equipment is used. The danger along this line is greatest with wood equipment, especially when the construction complicates the sterilization, as with churns. Also, the wood in churns may be in such a condition, because of cracks, surface irregularities, etc., that sterilization is difficult. With inadequate sterilization of equipment, organisms added to it from water supplies may grow at various points and a heavy contamination occur in this way. Apparently, extensive growth of organisms originally waterborne occurs most readily in wood churns and foci of contamination can be established there.

With milk, cream, ice cream, and comparable products, the danger that organisms from the water supply will be included is relatively small. Water normally does not come in contact with these products and the equipment used for them is readily sterilized. Should organisms be included, the usual holding conditions greatly limit their growth in milk and cream and prevent it in ice cream. With butter and various cheeses, water may be used directly to wash the product, standardize the composition, etc., so that organisms in the water supplies are easily added. As far as spoilage of the product is concerned, the danger is greatest with butter. Various bacteria that cause spoilage in butter are frequent in water and there are often opportunities for bacterial growth in butter. With cheeses, the rapid acid development during making and ripening tends to prevent growth of acid-sensitive organisms so that there is less tendency for spoilage from water-borne organisms than with butter; however, such spoilage is by no means excluded and often the temperature at which cheese is held permits active growth of organisms that tolerate the other conditions.

Water supplies that are satisfactory from the standpoint of public health may not be satisfactory from the standpoint of organisms that can cause spoilage in butter, cheeses, etc. The bacteriological methods used in establishing the safety of a water supply for drinking purposes ignore many of the species which can spoil dairy products so that approval of a supply by public health officials means relatively little so far as its use in washing butter, etc., are concerned. Outbreaks of spoilage in butter have been traced to city water supplies that analyses indicated were entirely satisfactory from the public health standpoint.

In a dairy plant a cross connection between a controlled and an uncontrolled water supply is a definite hazard that should be eliminated because of the possibility of objectionable water getting into the regular supply. Valves that are opened through error, leaky valves, etc., may cause difficulties in this way. In some instances, employees detect some irregularity by the flavor of the water. The dangers inherent in cross connections commonly are recognized in requirements set up for water supplies.

The bacteriological quality of a water supply may vary from time to time so that proper supervision requires rather frequent examinations. Variations are of special importance in untreated supplies coming from shallow wells but also involve other types of supplies.

Water as a source of butter spoilage organisms. Early investigations dealing with the bacteriology of butter indicated that organisms coming from contaminated water supplies sometimes cause spoilage in butter. *Ps. fluorescens* received special attention in this connection, perhaps because it is cultivated easily and because it produces rancidity in butter through hydrolysis of fat. It was isolated from water supplies used in butter plants, and various outbreaks of spoilage were attributed to it.

Outbreaks of cheesiness, surface taint, and rabbito have been traced to contaminated water supplies more frequently than outbreaks of other defects. Herreid *et al.*[1] investigated a plant that had difficulty with cheesy and putrid flavors in butter over a period of 3 yr and found that infected water caused the difficulty; the defect disappeared when a satisfactory water supply was provided. Cullity and Griffin[2] reported that outbreaks of rabbito encountered by various factories could be traced to defective water in every instance and with improved water supplies the irregularities disappeared. Sorensen[3] examined samples from 22,060 churnings of commercial butter and visited the plants producing butter of poor keeping quality. Putrid and cheesy defects were the ones most frequently encountered, and contaminated water was their main source. Rancid flavors were an important cause of poor keeping qualities and they were most frequently traced to equipment, especially churns. Poor keeping qualities due to organisms from contaminated water were eliminated by treatment of the water with hypochlorites. Pont[4] reported that the original source of rabbito infection in butter plants appeared to be the water supply.

In various additional investigations flavor defects in butter have been traced to contaminated water supplies. The types of organisms most often responsible are soil and water forms; they include especially organisms belonging to the genus *Pseudomonas* and their ability to grow at relatively low temperatures is significant because of the conditions under which butter often is held.

In experimental churnings there have been many instances of butter spoilage as a result of washing butter with water inoculated with such species as *Ps. putrefaciens, Ps. fragi, Ps. mephitica,* etc. Perhaps the most convincing evidence that contaminated water supplies can cause flavor defects in butter comes from those plants that regularly have spoilage unless the water is treated, usually with chlorine. In some instances neglect of one lot of wash water results in rapid spoilage of the churning involved.

Specific butter spoilage organisms from water. The organisms in plant water supplies that cause spoilage in butter have often been isolated. Linneboe[5] studied the water supplies of 52 butter plants; 9 contained *Ps. putrefaciens* as the water left the wells and 5 additional as the water left the holding tanks. Of 55 farm-water samples tested, 6 contained the organism. *Ps. putrefaciens* was detected in water coming from a plant well 237 ft deep that was drilled through clay and limestone and had the casing well above ground and set in cement. Long and Hammer[6] found *Ps. putrefaciens* widely distributed in water, including that from streams, lakes, roadside pools, and butter plants. Water from 29 plants was examined and samples from 14 yielded *Ps. putrefaciens* after enrichment in litmus milk. In 8 of the 29 plants the water was suspected of causing poor keeping qualities in the butter and *Ps. putrefaciens* was isolated from water of 5 of the 8 plants. With 436 water samples from 70 plants, Corley *et al.*[7] isolated *Ps. putrefaciens* from 22 (5%), representing 14 plants; 105 of the samples yielded typical fluorescent colonies and most of them produced spoilage when added to butter wash water. *Ps. fragi, Ps. graveolens,* and *Ps. mephitica* were obtained from certain samples. White[8] isolated *Ps. putrefaciens* from a dug well water supply that otherwise appeared to be of good quality.

Reynolds and Thornton[9] found two cultures of *Fl. maloloris* among 589 isolations of yellow-colony-producing bacteria from 140 samples of Alberta water. Wolochow *et al.*[10] reported that *Ps. fluorescens* was widely distributed in Alberta plant water supplies. Morrison and Hammer[11] isolated *Ps. fragi* from 3 (9.7%) of 31 samples of dairy-plant water.

Investigations in various commercial butter plants have resulted in the isolation from the water supplies of organisms responsible for butter spoilage. Commonly, such isolations have been prompted by consumer complaints and have been followed by treatment procedures for the contaminated supplies.

Water as a source of cheese spoilage organisms. Organisms that can cause spoilage in certain cheeses are common in water supplies and contaminated water must be looked upon as a possible source. Undoubtedly, the danger is greatest with the cheeses for which the curd is washed directly. During the ripening of cheeses, conditions permit development of the normal species of organisms so that multiplication of objectionable types also may occur unless they are restrained by such factors as *p*H, salt content, etc., to which the normal species are relatively resistant. Presumably, the acid that develops rapidly during the making and early curing of various cheeses is of most significance in this connection but other factors also are involved. With relatively unfavorable conditions for many species, it is probable that considerable numbers of organisms must be added to a cheese for their growth to be pronounced enough for spoilage to occur.

With cottage cheese, washing of the curd is extensive so there is abundant opportunity for its contamination from organisms in the water. The high acidity in the curd presumably prevents growth of certain species that may come from the water, but the limited keeping qualities of the cheese show the great dangers of contaminating cottage cheese with objectionable species. Although the most frequent types of spoilage in cottage cheese appear to be due to acid-resistant organisms, particularly yeasts and molds, other types sometimes are encountered. For example, a fruity flavor in cottage cheese has been traced to *Ps. fragi,* an organism that can cause a fruity odor in milk, cream, etc. It grows rapidly at relatively low temperatures, such as those normally used for holding cottage cheese.

Specific cheese spoilage organisms from water. *Escherichia-Aerobacter* organisms, which are the primary cause of gas formation in various cheeses, often are found in water supplies. In the examination of butter-plant water, Corley *et al.*[7] found them frequently. They regularly were absent in the supplies of some plants, they regularly were present in the supplies of other plants, and they sometimes were present in the supplies of still other plants. As the coliform organisms became more numerous in water, the total bacterial counts tended to be higher. During the examination of one sample of water from each of 70 plants, 51 isolations of coliform organisms were made, each being the main colony type on a plate streaked with lactose broth that developed gas following inoculation with 50 ml of water; 12 cultures (24%) were *E. coli,* 12 (24%) were *E. freundii,* 16 (31%) were *A. aerogenes,* and 11 (22%) were *A. cloacae.*

Hood and White [12] concluded that the bacteria which produce rancid flavors in cheddar cheese can come from various sources, including contaminated water. The nitrate-reducing organisms that appear to be of importance in causing discoloration in cheese when potassium nitrate is added to milk to control objectionable fermentations include various species that can come from water supplies.[13] *Ps. fragi,* which Hansen [14] found decreased the flavor score of cheese, may be present in water.

Not all the organisms that come from water supplies and develop in or on cheeses are objectionable and certain of them may be useful. *Bact. linens,* which is important on the surfaces of certain semi-hard and soft cheeses, has been found in surface water and undoubtedly is present in well water. Albert *et al.*[15] obtained it rather regularly from 17 samples of water used to water cows or found standing in stables or barn yards; it was recovered from 9 of 26 samples of water from streams, roadside pools, and a small lake.

General studies on butter-plant water supplies. In a number of instances the bacteriological quality of butter-plant water supplies in a given area has been investigated.

Wolochow *et al.*[16] studied 85 samples of water from 37 Alberta creameries. A large proportion of the samples were seriously contaminated with undesirable bacteria known to be injurious to the quality of butter. *Ps. fluorescens* was a very common contaminant. Results indicated that bacterial contamination that is serious from a quantitative standpoint is likely to be undesirable in qualitative aspects. The authors suggested that a short cut to solution of the problem of creamery water supplies may be to assume that all supplies are likely to contain undesirable bacteria and therefor should receive bactericidal treatment.

Study of the water supplies of 42 North Dakota creameries by Jensen [17] revealed that all but 4 contained one or more types of organisms capable of bringing about decomposition of fats and proteins. The organisms usually caused flavor defects in butter when introduced through the wash water. The defects were of various types, with cheesiness being the most common.

Corley *et al.*[7] found, in detailed investigations of water supplies of 70 Iowa butter plants, that some supplies regularly were satisfactory, some regularly were unsatisfactory, and others varied in quality from one examination to another. Some supplies that were acceptable from a public health standpoint were not suitable for use in butter manufacture because of the presence of butter spoilage species. When butter was washed with water known to contain coliform organisms, these organisms regularly were found in 1 ml of unsalted butter and usually in 0.1 ml; they sometimes were found in these amounts of salted butter.

Water storage tanks. Although many water tanks are used without the bacteriological quality of the water being significantly influenced, water coming from such tanks sometimes is poorer in quality than the water going into them. In an early study on the bacteria in butter wash water, Melick [18] found that water from melted ice, even when held at 10 C (50 F), may become filled with bacterial growth if allowed to stand a few hours in a wood tank that has not been thoroughly cleaned. Draining and thorough cleaning of a tank reduced the bacterial count on the water supplied by the tank from 100,000 per milliliter to 180. More recent studies [5,7] show that water tanks may be sources of coliform and/or butter spoilage species.

Presumably, wood tanks are more often a source of butter spoilage organisms than metal tanks. Organisms falling into a poorly covered tank may be responsible for such contamination, but growth also is involved, particularly when there is an opportunity for organic matter to accumulate in the tank.

Castell and McDermott[19] found that water from several hundred deep and shallow wells, having low initial counts, contained organisms that multiplied extensively when the water was enclosed in a container. Type and size (up to 80 gal) of the container had relatively little effect on the numbers of bacteria that developed. The organisms included mainly non-saccharolytic, Gram-negative types having the general characters of the genera *Alcaligenes, Pseudomonas,* and *Achromobacter.* A large proportion of them were lipolytic, proteolytic, and produced oxidase; many were active at low temperatures. Inoculation of sterile tap water with pure cultures appeared to confirm the observation that growth in water of low organic content was restricted to certain groups of Gram-negative bacteria, *Pseudomonas* species being particularly active.

A water storage tank should be recognized as a possible source of organisms in a water supply and adequate protection and care should be given it. Tests, from the bacteriological standpoint, of the water going to and coming from the tank, are advisable. The build-up of organisms in a tank is influenced by various factors; some of these are obvious, whereas others apparently are unknown.

Attempts to detect butter spoilage organisms in water. Most of the studies on spoilage organisms in dairy-plant water have been carried out from the standpoint of butter because the dangers are greatest with this product. Quantitative as well as qualitative considerations are involved since various factors influence growth of organisms in butter, and wash water may contain spoilage organisms without causing deterioration. In salted butter with certain physical properties, heavily contaminated wash water may not cause spoilage. Even with unsalted butter having physical properties that favor growth of organisms, considerable numbers of spoilage types may be required to cause a defect.

Butter washing tests. Since contaminated water may be especially harmful when used to wash butter, one type of test employed on water involves actual washing of small lots of experimental unsalted butter (made from pasteurized cream with sterile equipment) with the water in question, and holding of the butter at favorable growth temperatures, such as 21.1 C (70 F). Rapid spoilage of the butter, when the control butter washed with water from the same lot after sterilization keeps satisfactorily, is convincing evidence of the danger involved. Delayed spoilage may be due to irregularities of one type or another and is much less convincing than rapid spoilage. Organisms getting into the butter from the air during the long exposure while it is being washed, worked, etc., may be important, particularly with the poor moisture distribution obtained in most lots of butter worked by hand. In general, butter washing tests have been helpful in the detection of butter spoilage organisms in water, especially when the species involved grow poorly, or not at all, on the media used to plate the water.

Plating procedures. Because the organisms causing spoilage in butter vary greatly in colony type, action on protein, action on fat, etc., and also because non-spoilage types frequently show characters essentially the same as those of certain spoilage types, detection of spoilage organisms by the plating of water presents many difficulties, even with differential media. The problem is further complicated by the fact that such an important spoilage species as *Ps. putrefaciens* does not initiate growth readily on media, including those especially favorable for it.

In general, actively proteolytic and/or lipolytic bacteria are objectionable in a water supply. However, it should be recognized that not all organisms showing one or both of these characters produce changes in butter. With 64 oxidase-positive and 28 oxidase-negative cultures, Trembath [20] found that the former produced spoilage in butter much more commonly than the latter, but that some oxidase-positive types failed to cause spoilage and some oxidase-negative types produced defects.

Plating of water samples, particularly on a medium permitting detection of proteolysis and lipolysis, supplies useful general information with reference to a water supply and is especially helpful in detecting changes in the bacteriological condition of a supply. The plates should be incubated at relatively low temperatures, such as 21.1 C (70 F), since certain significant organisms fail to grow at somewhat higher temperatures. By incubating plates at various temperatures additional information may be obtained. Jezeski and Macy [21] reported that some samples of creamery water yielded bacterial counts of considerable magnitude when the plates were incubated at 8 C (46.4 F); this indicates that creamery water supplies may contain organisms capable of growth at relatively low temperatures.

Significance of total plate counts. It would be expected that the higher the bacterial count on butter wash water, the greater the danger from it. This general relationship is shown by the following data reported by Corley *et al.*[7] The counts were made on standard agar for milk analysis, the plates being incubated 4 days at 21 C (69.8 F), and the effect of the water on butter washed with it was tested in experimental churnings, each involving about 1 pt of cream; the butter was unsalted.

GENERAL RELATIONSHIP BETWEEN NUMBERS OF BACTERIA IN BUTTER WASH WATER AND PRODUCTION OF FLAVOR DEFECTS IN THE UNSALTED BUTTER

Bacterial Counts on Water *Group*	 *Range per Milliliter*	No. Samples in Group	Samples of Water Producing Flavor Defects in Butter in 4 Days or Less (Per Cent)
a	<1 to 50	204	7
b	51 to 100	43	23
c	101 to 200	52	44
d	201 to 300	31	32
e	301 to 400	22	50
f	401 to 750	35	47
g	751 to 1,500	36	61
h	over 1,500	13	69

The results indicate that a small percentage of low-count samples caused spoilage and some of the high-count samples failed to cause spoilage. The former irregularity presumably was due to failure of certain species important in butter spoilage to develop colonies on the plates; the latter irregularity evidently was because certain species of bacteria do not cause spoilage even when present in considerable numbers. The data definitely indicate that the dangers of causing spoilage in butter are greater with relatively large numbers of organisms in the wash water than with small numbers. Accordingly, total counts can be employed as a basis for rejection of water for use in dairy plants. Since proteolytic and/or lipolytic organisms undoubtedly are more objectionable than types showing neither of these characters, requirements that take them into account can be included.

Bacterial standard for dairy-plant water supplies. Bacterial standards for dairy-plant water supplies should recognize the two types of organisms that are objectionable from the standpoint of dairy products: (1) those of public health significance, and (2) those that may be important in causing spoilage in dairy products. Although spoilage due to contamination from water supplies is less common with various other dairy products than with butter, there is little justification for using supplies in the preparation of such products that would not be acceptable in butter plants. Moreover, contamination of market milk, market cream, etc., with the types of organisms involved in butter spoilage may cause deterioration in them should the holding conditions be favorable for growth as a result of carelessness in the producing plants, distribution centers, homes, etc. Spoilage of market milk and cream with such typical soil and water organisms as *Ps. fragi, Ps.*

fluorescens, etc., is rather common, even with moderate holding periods, and the organisms may have come from the water supplies.

Corley *et al.*[7] proposed essentially the following standards for butter-plant water supplies and suggested that they probably also should be applied to all types of dairy plants.

1. The water should meet all drinking water standards.
2. The total bacterial count on standard agar for milk analysis containing fat emulsion, with incubation 4 days at 21 C (69.8 F), should not exceed 100 per milliliter and preferably should be under 50.
3. If proteolytic and/or lipolytic bacteria are present, the numbers should be relatively low, and actively proteolytic or lipolytic organisms, as indicated by the colony types, should not exceed 5 per milliliter.
4. When the water is used to wash butter in experimental churnings, the unsalted butter should keep satisfactorily for at least 4 days at 21 C. Experimental churnings are not needed in regular examinations of water supplies but are advisable when the quality of the water is still in doubt after the usual bacterial tests or when a supply definitely is suspected of causing deterioration in butter.
5. A water supply should be examined at regular intervals; if it commonly is satisfactory, examinations can be less frequent than if it is rather variable in quality.

Water treatment. Recognition of the role of contaminated water in the spoilage of certain dairy products has led to various suggestions with reference to the treatment of water of unsatisfactory bacteriological quality. For the most part the treatment has been considered necessary because of the presence of spoilage organisms in the water, but if species of public health significance are present they also will be included.

Pasteurization and recooling of water has been advocated and undoubtedly is effective. Pasteurization and recooling may be applicable to a small operation as an emergency procedure but is expensive and inconvenient; for a large operation it is impossible without special equipment and even then the expense eliminates it as a practical procedure. Filtration through special filters, light treatment, ozone treatment, various chemical treatments, etc., also have been proposed. Treatment with chlorine is the most widely used procedure and its effectiveness, low cost, convenience, etc., have been thoroughly established. Various types of equipment have been developed for introducing the chlorine, in one form or another, into the water. The extensive use of chlorine in the treatment of city-water supplies is ample evidence of the value of the process.

Sediment in water. In some instances water contains excessive amounts of sediment which can be added to a dairy product directly, or which is left in equipment and then gets into a product when the equipment is used. Water of this type may or may not be satisfactory, bacteriologically. Filters of various types commonly are employed to remove such material; when used as the water is flowing from pipe lines into churns, vats, etc., they are not always effective because, with the speed at which the filter must operate, removal of finely divided material is difficult. Filtration or sedimentation of water going into a storage tank has its advantages with special equipment which can operate effectively at the rate the water comes from the well.

Because of the attention given extraneous matter in dairy products, sediment in water should be removed as completely as possible. Windblown material that gets into water directly or by first settling into open equipment also should be considered.

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